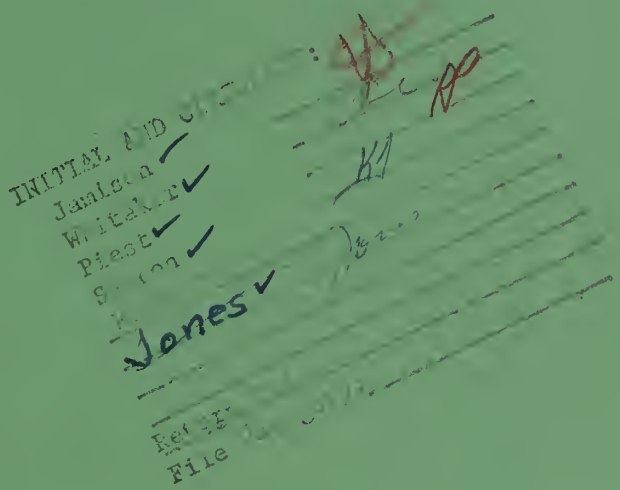
RESEARCH IN PLANT TRANSPIRATION: 1962

Production Research Report No. 87



Agricultural Research Service

U.S. DEPARTMENT OF AGRICULTURE

in cooperation with

Georgia Agricultural Experiment Stations

and

Meteorology Department

U.S. Army Electronics Research and Development Activity

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BY JAMES E. PALLAS, Jr., ANSON R. BERTRAND, DONALD G. HARRIS,
CHARLES B. ELKINS, Jr., AND CLYDE L. PARKS

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WASHINGTON, D.C. ISSUED MARCH 1965

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C., 20402 - Price 45 cents

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RESEARCH IN PLANT TRANSPIRATION: 1962

By James E. Pallas, Jr., *research plant physiologist,* and Anson R. Bertrand, Donald G. Harris, Charles B. Elkins, Jr., and Clyde L. Parks, *soil scientists, Soil and Water Conservation Research Division, Agricultural Research Service*

The purpose of this research in plant transpiration is to evaluate the function of plants in the hydrologic cycle. Specifically the objectives are as follows:

(1) To measure the effects of radiant energy, temperature, humidity, and other environmental factors on transpiration.

(2) To develop better methods and techniques for measuring transpiration.

(3) To gain knowledge of the cellular processes and factors that control or affect guard cell action.

(4) To observe and to measure accurately the reactions of guard cells.

(5) To discover and to evaluate chemical, genetic, physical, and other means to control transpiration.

(6) To develop instruments and equipment that will aid in fulfilling all these objectives.

Moisture is transmitted from the land surface to the atmosphere by evapotranspiration. Evaporation of moisture from a nonvegetated soil surface accounts for a part of this moisture transfer and is controlled by micrometeorological and soil factors. Where vegetation is present, the biologic properties of the plant, as affected by the environment, control the transfer of water from the soil to the atmosphere. Transpiration is largely controlled by plants through manipulation of the guard cells surrounding the stomatal openings in the leaves.

An understanding of the mechanism of stomatal opening and closing and of the reaction of plants to certain external stimuli will aid in the development of control procedures and will ultimately lead to increased water use efficiency.

This report, like the previous ones (*51*, *75*),[1] is divided into three complementary areas of research; namely the effect of environmental factors on transpiration, cellular studies for elucidating the mechanism of guard cell action, and the testing of chemicals that indicate some potential in controlling transpiration.

CONTROLLED ENVIRONMENT STUDIES

Light Distribution in Controlled Environment Growth Room

A study was undertaken to characterize the distribution of radiant energy under the high intensity lighting system used in the controlled environment growth room at Watkinsville, Ga. The source of light for each of three plant growth areas, or bays, within the growth room is a battery of 96 incandescent bulbs.[2] The bulbs are located 9 inches above the ceiling of the room and the light is filtered through approximately 4 inches of flowing water and one-half inch of glass before entering the room. The bulbs are arranged in 8 rows of 12 bulbs each and are spaced 6 inches apart on center in both directions.

The dimensions of the glass openings are 4 by 6 feet. Half of the bulbs, alternately spaced, are controlled by a variable transformer so that light intensities can be varied from zero to one-half of maximum intensity. The other half of the bulbs are controlled by a circuit breaker and are either on or off. Intensities between one-half and full capacity are obtained by having the fixed voltage bulbs on and by adjusting the variable voltage bulbs from zero to full intensity. For further details, refer to the first annual report (*75*, *pp. 8–10*).

Procedure

Light intensity, or radiant energy, was measured over a 5- by 7-foot area on a 6-inch grid consisting of 11 rows and 15 columns. This area extends 6 inches beyond the edge of the glass on all sides and represents the maximum area likely to be used for plant growth.

[1] Italic numbers in parentheses refer to Literature Cited, p. 51.

[2] Only Sylvania 300-watt reflector bulbs with medium skirted base have been evaluated. Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

All data were collected with a Beckman-Whitley Model H 188–01 radiometer at a distance of 96 cm. from the glass. Several combinations of spot and flood bulbs were tried, and the distribution of light from seven combinations is included in this report. The seven bulb combinations used are summarized in table 1.

TABLE 1.—*Combinations of 300-watt reflector spot and flood bulbs used in light distribution study*

Bulb code No.[1]	Bulbs used		Replications
	Spot	Flood	
	Number	*Number*	*Number*
1	48	0	3
2	48	0	1
3	96	0	3
4	96	0	1
5	0	48	1
6 [2]	32	16	1
7 [2]	80	16	1

[1] All combinations are referred to by code number in this report.
[2] See figure 38 in appendix for arrangement.

Results and Discussion

Average light intensity, or radiant energy, and uniformity of distribution were found to be dependent on the size of the area included in the analysis, as shown in tables 2 and 3.

Table 2 indicates that the average intensity of radiant energy, expressed as calories per square centimeter per minute (cal. $cm.^{-2}$ $min.^{-1}$), increases as the area size is reduced from 35 to 15 square feet. The intensity tends to level out for areas less than 15 square feet, except for bulb code 5 when all flood bulbs were used.

In table 3 the coefficient of variation of radiant energy drops sharply for all bulb combinations as the area size is reduced from 35 to 15 square feet. Further reductions in area size do not appreciably reduce the variance. Consequently, it seems advisable to confine experimental plants to no greater than a 3- by 5-foot area. In table 3 the sharp increase in the coefficient of variation for areas greater than 15 square feet is probably due to sharp reductions in peripheral intensities starting at the edge of the glass.

Figure 1 illustrates these border effects on light intensity. The average intensities of eleven 5-foot rows parallel to the 6-foot edge of the glass (fig. 1, *A*) and of fifteen 3-foot columns parallel to the

TABLE 2.—*Average intensity of radiant energy as affected by size of growth area*

Bulb code No.	Intensity (cal. $cm.^{-2}$ $min.^{-1}$) for indicated area (feet)[1]					
	5 by 7	4 by 6	3 by 5	2 by 4	2 by 3	2 by 2
1	0.33	0.41	0.48	0.51	0.51	0.50
3	.68	.84	.98	1.04	1.03	1.03
5	.30	.35	.39	.43	.44	.45
6	.38	.44	.48	.46	.45	.46
7	.66	.79	.88	.90	.89	.87

[1] First digit for area represents dimension parallel to 4-foot width of glass and second digit represents dimension parallel to 6-foot length of glass.

TABLE 3.—*Coefficient of variation of radiant energy of individual observations as affected by size of growth area*

Bulb code No.	Coefficient of variation for indicated area (feet)[1]					
	5 by 7	4 by 6	3 by 5	2 by 4	2 by 3	2 by 2
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	47	27	12	9	8	8
2	49	27	12	10	10	8
3	46	26	10	7	7	7
4	45	24	9	7	7	8
5	34	21	14	10	7	6
6	34	18	11	10	9	8
7	37	19	7	6	6	4

[1] First digit represents dimension parallel to 4-foot width of glass and second digit represents dimension parallel to 6-foot length of glass.

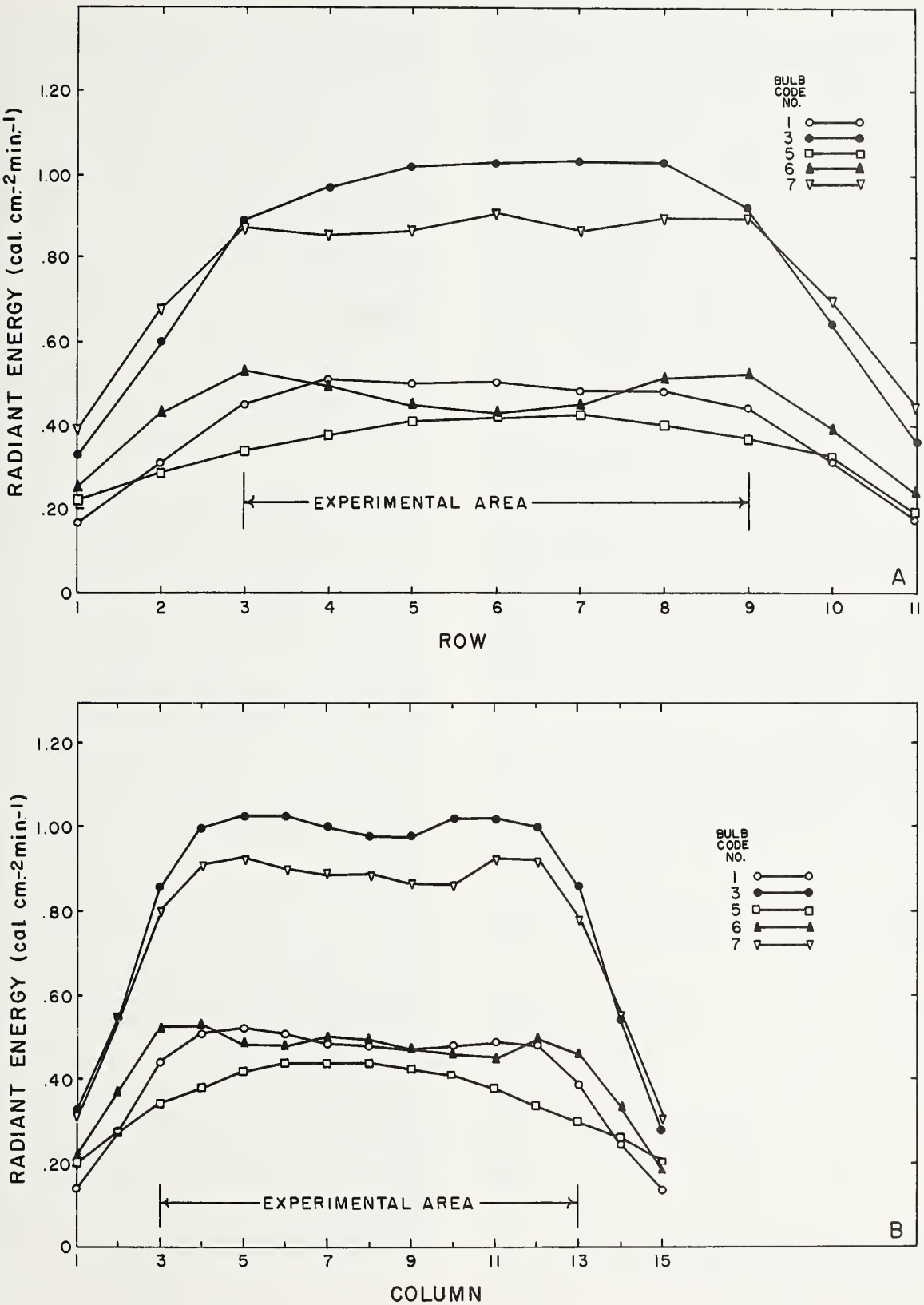

FIGURE 1.—Average radiant energy for (*A*) eleven 5-foot rows and (*B*) fifteen 3-foot columns of grid under five combinations of spot and flood bulbs, as indicated by bulb code numbers.

4-foot edge of the glass (fig. 1, *B*) are shown. Five-foot rows (11 observations) and three-foot columns (7 observations) were chosen in view of the decision to confine experimental plants to a 3- by 5-foot area. Rows 1 and 11 and columns 1 and 15 represent intensities 6 inches beyond the edge of the glass, rows 2 and 10 and columns 2 and 14 represent intensities at the edge of the glass, and rows 3 and 9 and columns 3 and 13 bound the limits of the area considered acceptable for experimental purposes.

Data in figure 1 support the earlier conclusion that experimental material should be confined to a 3- by 5-foot area. They also illustrate that intensity plateaus extending to within 6 inches of the edge of the glass are obtainable.

The coefficients of variation in light intensity for the seven 5-foot rows and eleven 3-foot columns, which are within the experimental area in figure 1, are listed in table 4. Table 4 also includes the coefficients of variation for the light intensity among the fifteen 1-square-foot areas, which comprise the 3- by 5-foot experimental area. These data indicate that the bulb arrangement of code 7 is superior to all others investigated. The coefficient of variation of 2.4 percent between the fifteen 1-square-foot areas was at least 50 percent less than any other arrangement. It is also considerably less than the biological variations that are normally encountered. Consequently, the uniformity of light distribution should not be the factor limiting precision of transpiration data for plant systems that occupy at least 1 square foot of bench space.

Data based only on spot bulbs indicate that uniformity of light distribution is relatively independent of intensity. A comparison of bulb codes 1 and 3 suggests this fact, and additional data at lower intensities substantiate the observation. All data reported in the tables were collected at 96 cm. from the glass ceiling. One test at 74 cm. resulted in distribution patterns and variances that were similar to those obtained at 96 cm. The effect of orienting bulb sockets (*53*) on distribution was examined for four patterns. In general, the coefficient of variation for a 4- by 6-foot area can only be reduced from about 26 to 23 percent. The coefficient of variation for all smaller areas increased to approximately 15 percent. Consequently, bulb orientation is not considered practical.

In summary then, uniformity of light distribution from incandescent reflector bulbs was found to be dependent on the type of bulb combination used and the size of the area. It was relatively independent of intensity and distance from the source. Orientation of bulb sockets was not beneficial. The maximum area under each bay for growth room experiments should be 3 by 5 feet. A combination of flood and spot bulbs, ratio 1 to 5, resulted in the best distribution of light.

TABLE 4.—*Coefficients of variation in light intensity for rows, columns, and fifteen 1-square-foot areas within experimental area under 7 bulb combinations*

Bulb code No.	Rows [1]	Columns [2]	Fifteen 1-sq.-ft. areas [3]
	Percent	*Percent*	*Percent*
1	5. 5	7. 7	----------
2	7. 0	7. 9	7. 0
3	6. 0	6. 4	----------
4	5. 8	5. 3	5. 1
5	8. 6	12. 0	10. 5
6	8. 5	5. 1	7. 1
7	2. 2	5. 4	2. 4

[1] Seven 5-foot rows (3 through 9).
[2] Eleven 3-foot columns (3 through 13).
[3] Intensity for square foot is equal to average of 9 observations on 3 by 3 grid.

Radiant Energy and Soil Moisture Tension Effects on Transpiration, Guard Cell Activity, Leaf Temperature, and Photosynthesis of Corn Plants

Radiant energy is one of the most important factors affecting transpiration under a controlled environment (*51*). These findings are in agreement with field observations (*52*, *68*, *69*). However, the effect of radiant energy on heat exchange of the plant with its environment, stomatal behavior, and water use economy is little known.

The diversities in the morphology, anatomy, and physiology of land plants would lead one to suspect that in their reaction to radiant energy wide differences could exist between different species and possibly even between different varieties. Therefore it is necessary to select representatives of many species of plants for transpiration studies under controlled conditions before one can understand and adequately describe plant-water relations in the continuum of soil-plant-air. It is anticipated that both monocotyledonous annuals and woody dicotyledonous perennials will be studied, with an initial range from pasture to row crop to forest species.

Studies with these plants, because of their diverse nature, should serve as benchmarks in working toward understanding and eventual control of the transfer of water from plants to the atmosphere. More efficient use of the radiant energy reaching the surface of the earth might also be realized through these studies.

The science of plant breeding has developed more adaptable, higher yielding, and disease- and insect-resistant varieties, but our understanding of

the physiological and biochemical reasons for these improvements has developed very slowly—indeed, it is still embryonic. The future of plant science and, to a degree, the well-being of mankind depends on increased understanding of the physiological and biochemical responses of plants to their environment.

Corn was chosen first for these studies because of its economic and probable micrometeorological importance. Its widespread culture and large acreage can be considered to contribute significant quantities of water vapor to the atmosphere. The growth and culture of corn in the field have been studied extensively; however, studies of transpiration under controlled conditions have not been reported.

Procedure

The test plant *Zea mays* L. variety Dixie 82 is a corn hybrid adapted to the Piedmont Plateau region of the Southeastern United States. Environmental conditions for this work were selected as representative of the Piedmont by determining the average day and night temperatures and relative humidities from 20 years of meteorological records of the Athens, Ga., area during the early corn growing season. The standard procedures as described below were used throughout this work.

Corn seeds were germinated in vermiculite at 30° C. and transplanted 24 hours after sowing into 1,663 gm. of Cecil sandy clay loam soil contained in 46-ounce asphalted juice cans. The cans were fitted with Selas porous ceramic plate disks, 3 by ¼ inches, embedded in plastic (see fig. 2). This arrangement allowed for removal of excess soil water by applying a vacuum to the outlet tube. One hundred cans were routinely prepared so that further selection of test plants could be made during the first few days of growth; 67 plants were actually used.

The soil was fertilized with 4,000 pounds of 6–12–12 (120 p.p.m. of nitrogen (N), 103 p.p.m. of phosphorus (P), and 199 p.p.m. of potassium (K)) and a ton of dolomitic limestone per acre (1,160 p.p.m. of calcium carbonate ($CaCO_3$) and 740 p.p.m. of magnesium carbonate ($MgCO_3$)). "Krilium" was used at the rate of 1 pound per 350 pounds of soil to improve soil aggregation (*23*) in all experiments except the first.

To minimize variability that might be correlated with edaphic factors, a standard procedure of soil preparation was adopted. Soil was passed through a 10-mesh screen to remove trash. It was air-dried to 1- to 1.5-percent moisture on an oven-dry basis. Krilium and lime were mixed with the dry soil in a rotating drum mixer. Water was added to bring the moisture content to approximately the plastic limit, and again the soil was air-dried. Fertilizer was added and mixed with a rotary drum mixer just prior to each experiment.

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After transplanting, sufficient water was added to each can to approximate the 0.05-atmosphere soil moisture tension value as determined from soil moisture desorption curves (*56*). The cans then remained at 30° C. for a day. The coleoptile emerged the third day after sowing. At 8 a.m. of this day, the Sylvania VHO cool white fluorescent lamps were turned on, giving radiant energy of 0.6 to 0.4 cal. $cm.^{-2}$ $min.^{-1}$ at the soil surface. On the fourth day the plants were thinned to one plant per container and a plastic cover was set over the can, snug with the stem to minimize evaporation.

The containers and plants were transferred to the growth room (*75*) and held at full light intensity (0.85 cal. $cm.^{-2}$ $min.^{-1}$), 25° C., and 60-percent relative humidity for a 14-hour photoperiod and at 20° and 90-percent relative humidity for a 10-hour nyctoperiod. This cycle was repeated until the plants were 15 days old. The full light intensity is lower than that used in other studies (*51*, *75*), reported as 1.35 cal. $cm.^{-2}$ $min.^{-1}$. This lower value was a result of increasing the water barrier between the light and the room from the 2 inches previously used to 4 inches, and thus more long wave radiation was intercepted. To provide adequate water for growth, the plants were rewatered to near 0.05 atmosphere on the 5th, 9th, and 14th days of age.

To simulate field conditions throughout these studies, the lights were gradually raised to their required intensity from 6 to 8 a.m., and from 6 to 8 p.m. the intensity was gradually reduced to "off." To counteract light quality effects on growth (*22*) because of an increasing proportion of far-red as the growth room lights dimmed (*75*), the last 15 minutes of the photoperiod was supplemented by turning on slim-line cool white fluorescents to supply 0.1 cal. $cm.^{-2}$ $min.^{-1}$ of radiant energy.

Four separate plant populations were grown for the four experiments with corn. In general, the experimentation progressed as follows: From the 14th through the 20th day of age the effects of changing radiation patterns on transpiration were studied. On the 21st day all plants underwent the same environmental conditions, bringing them back to a common point. From the 22d day of age to termination of the experiment the plants went through an irrigation-drying-reirrigation cycle. For brevity in this report, the first 6 days are referred to as the preconditioning part, and the irrigation-drying-reirrigation cycle as the soil moisture tension part of each experiment.

Specifically, the experimental conditions were as follows: At 14 days of age 57 plants were selected for uniformity and randomly divided into three equal groups (A, B, and C). Starting at 6 a.m. on day 1 of the experiment, each group was subjected to different light conditions as outlined

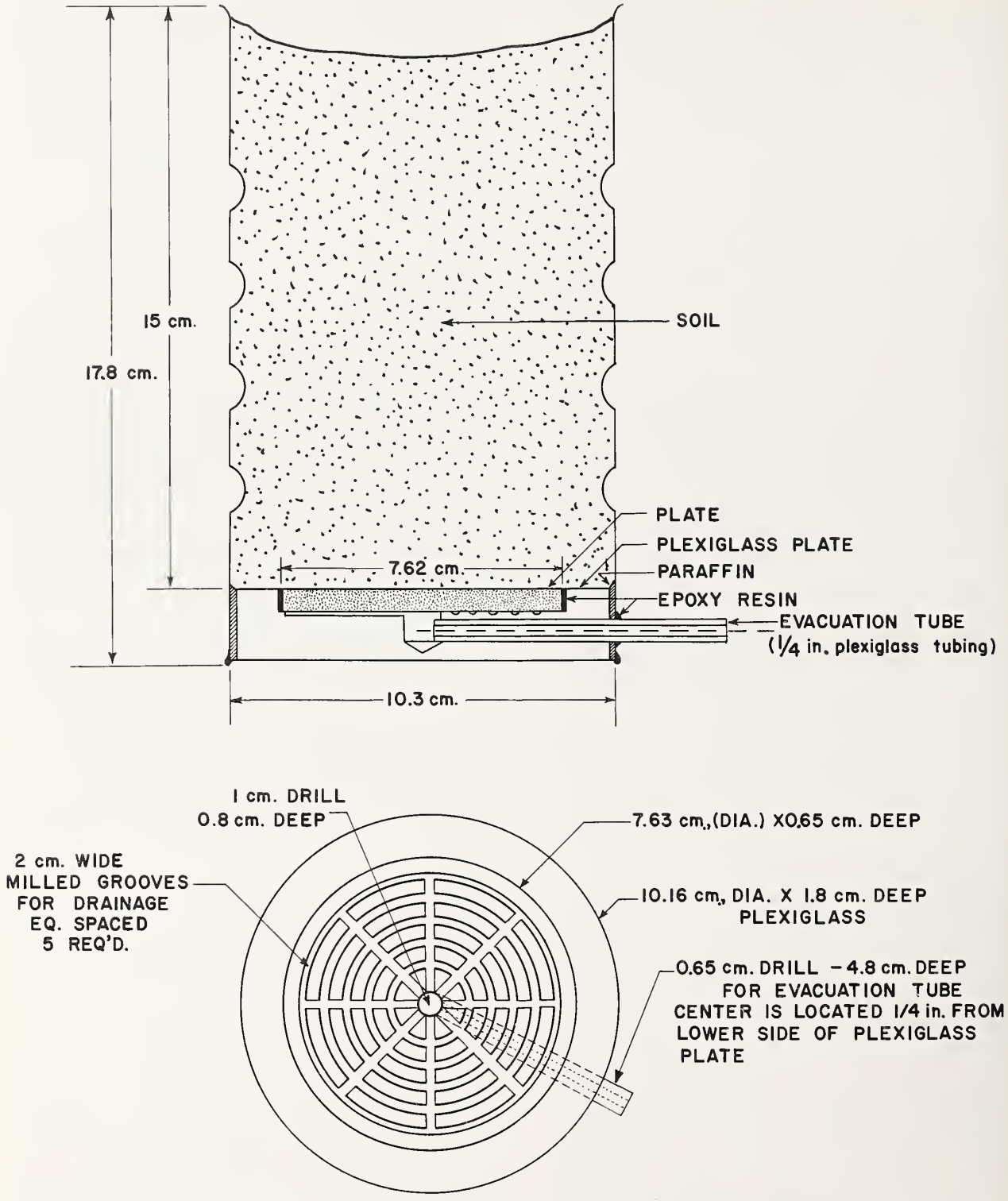

FIGURE 2.—Experimental container, providing for removal of soil moisture by suction.

TABLE 5.—*Location of plant groups (A–C) during experiment* [1]

Days	Bay 1	Bay 2	Bay 3
1 and 2	C	B	A.
3 and 4	A	C	B.
5 and 6	B	A	C.

[1] Bay 1, 2, and 3 had ¼, ½, and full sunlight equivalent, respectively, which equaled 0.19, 0.52, and 0.85 cal. cm.$^{-2}$ min.$^{-1}$.

in table 5. Beginning on day 1 and each day thereafter, starting at 6 a.m. and ending at 8 p.m., hourly weighings of the cans were made for determination of transpiration, which is expressed as grams per square decimeter of leaf area.

Two plants at each light intensity had copper-constantan thermocouples (40 gage) attached to the third from the topmost leaf on the upper and lower epidermis, midway on the blade (fig. 3), to determine leaf temperature (° C.). Care was taken to keep the sensing junction in intimate contact with the cuticle. The junction was, therefore, buried among the trichomes.

The photoperiod remained 14 hours and the day and night temperature and relative humidity conditions were as stated earlier. All plants were watered to near 0.05 atmosphere at 8 p.m. on days 2, 4, and 6, subsequent to shifting to the various bays.

By using an improved model of the stomata camera (*75*), guard cell activity was monitored visually (fig. 4) to establish trends of operation. The percentage of stomata open on upper and lower surfaces of the third youngest leaf midway between the tip and base was checked on two representative plants from each bay. All stomata on such leaves are operable. A minimum of 240 stomata were observed each hour.

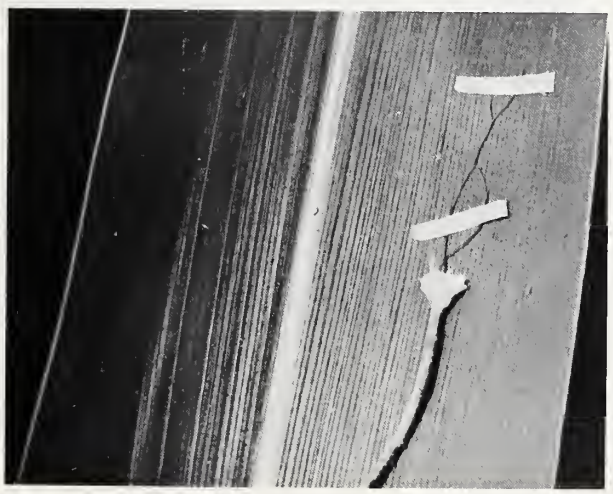

Figure 3.—Copper-constantan thermocouple attached to corn leaf for leaf temperature measurement.

Plant growth was estimated from leaf measurements made twice daily from 6 to 8 a.m. and 6 to 8 p.m. Earlier work had shown that length times width times 0.7 gives an accurate estimate of corn leaf area.

On day 7 (21 days of age) all plants received full light for 10 hours. This procedure was adopted to nullify any preconditioning effects. At 8 p.m. of this day the soil was watered to near 0.05 atmosphere. Ten representative plants were sacrificed to give an estimate of plant top and root weight. Up to day 8 all experiments were conducted according to the same plan.

During the soil moisture tension part of the experiments, beginning on day 8, conditions were as follows:

Experiment 1.—14 hours' light, 25° C., 60-percent relative humidity; 10 hours' dark, 20°, 90-percent relative humidity. Plants remained under one-fourth, one-half, or full sunlight equivalent with increasing moisture stress; rewatered at 4 to 8 atmospheres by irrigation to 0.05 atmosphere; non-Krilium-treated soil.

Experiment 2.—Same as experiment 1 except day relative humidity was 90 percent and irrigation near 15 atmospheres; Krilium-treated soil.

Experiment 3.—Same as experiment 1.

Experiment 4.—Same as experiment 2 except day relative humidity was 30 percent.

On the day of irrigation of each bay, after soil moisture drawdown, five plants were harvested for fresh and dry weights. Relative turgidity (*74*) measurements of corn leaves were also made in experiment 2. Two days after reirrigation of the low light bay, the experiment was terminated by harvesting all test plants to determine fresh and dry weights.

Limited measurements of photosynthesis were made during the soil moisture tension part by means of a Beckman infrared gas analyzer. The leaf chamber (fig. 4) used was fashioned after Brun's model (*13*). Photosynthesis was measured in experiment 1 on the day of rewatering plants under full sunlight equivalent; experiment 2, on the days of rewatering plants at each light intensity; experiment 3, from day 8 through rewatering of full sunlight plants and the day of rewatering, and the following day on the one-half sunlight equivalent population; and experiment 4, from day 8 through rewatering the one-half and one-fourth sunlight population, and the following day of the one-half sunlight population.

Data reduction and editing were accomplished with the 1620 computer. The following integral transformation equations were used:

$$(1)\quad ^{\circ}\text{C.}=0.254+25.86\ S^{0.971}-0.317\ S^{2}$$

where S=signal of copper-constantan thermocouple in millivolts.

$$(2)\quad VP_L=0.17075\ T+0.51176\ \frac{1}{(T)}+0.58782\ \ln T+3.16152_e{}^{0.5977\ T}+0.00396\ T^{2.4}+2.427$$

where VP_L=vapor pressure of water in millibars at leaf temperature and T=temperature in degrees centigrade.

$$(3)\quad \Theta_w=\frac{100\left[\begin{array}{c}\text{Total weight}-(\text{tare weight of}\\ \text{container}+\text{dry weight of soil}\\ +\text{green weight of plant})\end{array}\right]}{\text{Dry weight of soil}}$$

where Θ_w=percent moisture by weight.

Results and Discussion

Transpiration and leaf temperature of the corn plants in the preconditioning part were measurably affected by radiant energy, as shown in figures 5–8. Mechanical failure of growth room machinery caused the extreme deviations in transpiration, as indicated in figure 6 on day 1 at 2 p.m. and in figure 7 on day 1 at 8 and 9 a.m. and 1 p.m.

All plants received full sunlight in experiment 3 on day 6 (fig. 7) because of an error in programing. The decrease in transpiration between 10 a.m. and 8 p.m. resulted from increasing soil moisture tensions, as shown in table 6.

An extensive analysis of variance for the preconditioning part of experiments 2 and 4 indicated total transpiration was much the same in all four experiments on day 7 (figs. 9–12). Therefore, the sequence in which light intensities occurred (days 1–6) did not affect the transpiration on day 7. No significant difference in transpiration between plants in any one bay indicated that the plants were homogeneous when transpiration was ad-

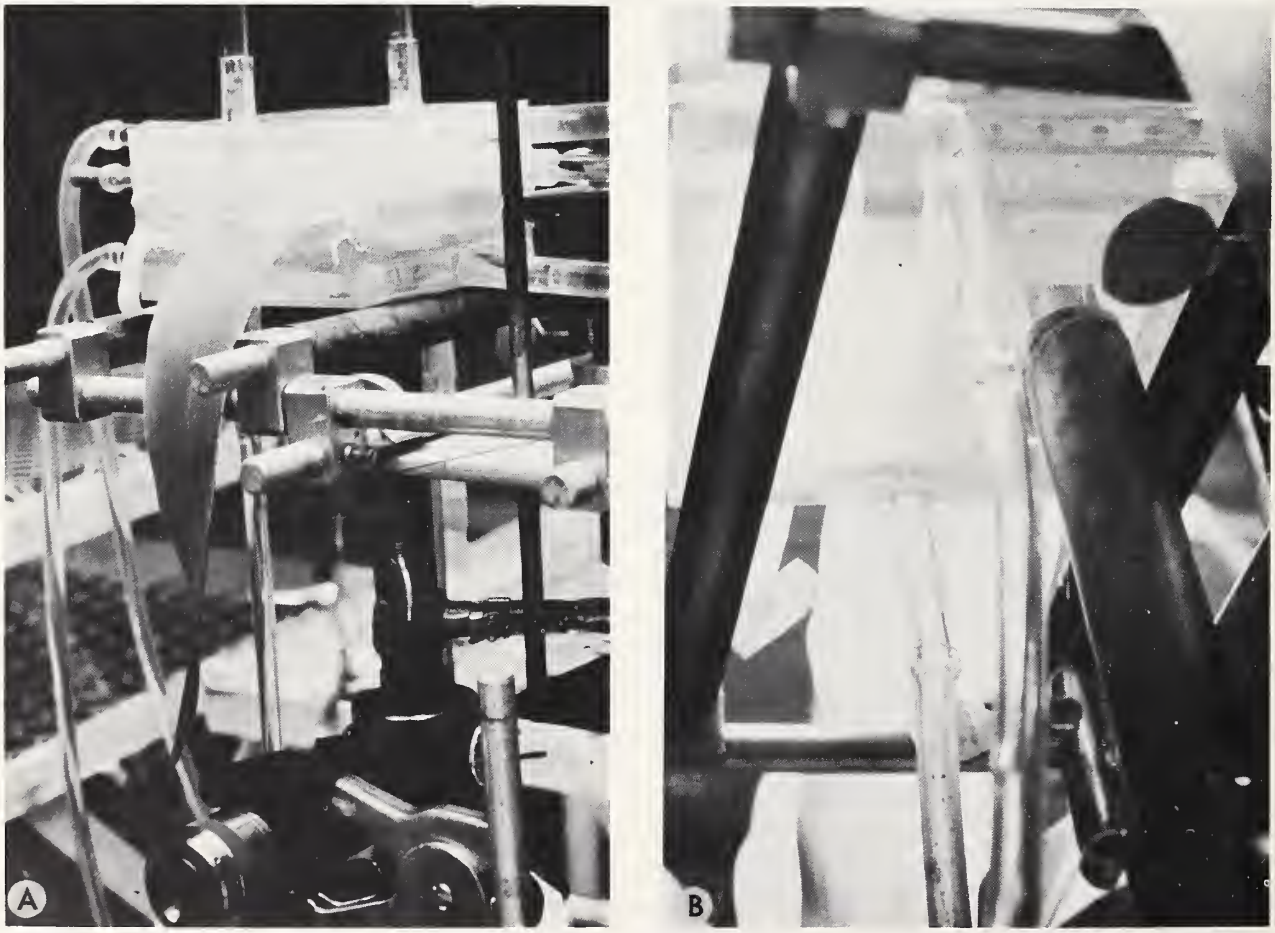

FIGURE 4.—*A*, Water-cooled leaf chamber for measurement of apparent photosynthesis of corn, concurrent with stomatal observations; *B*, underside of leaf chamber, showing indentation for stomatal observation with objective and microscope tube in place.

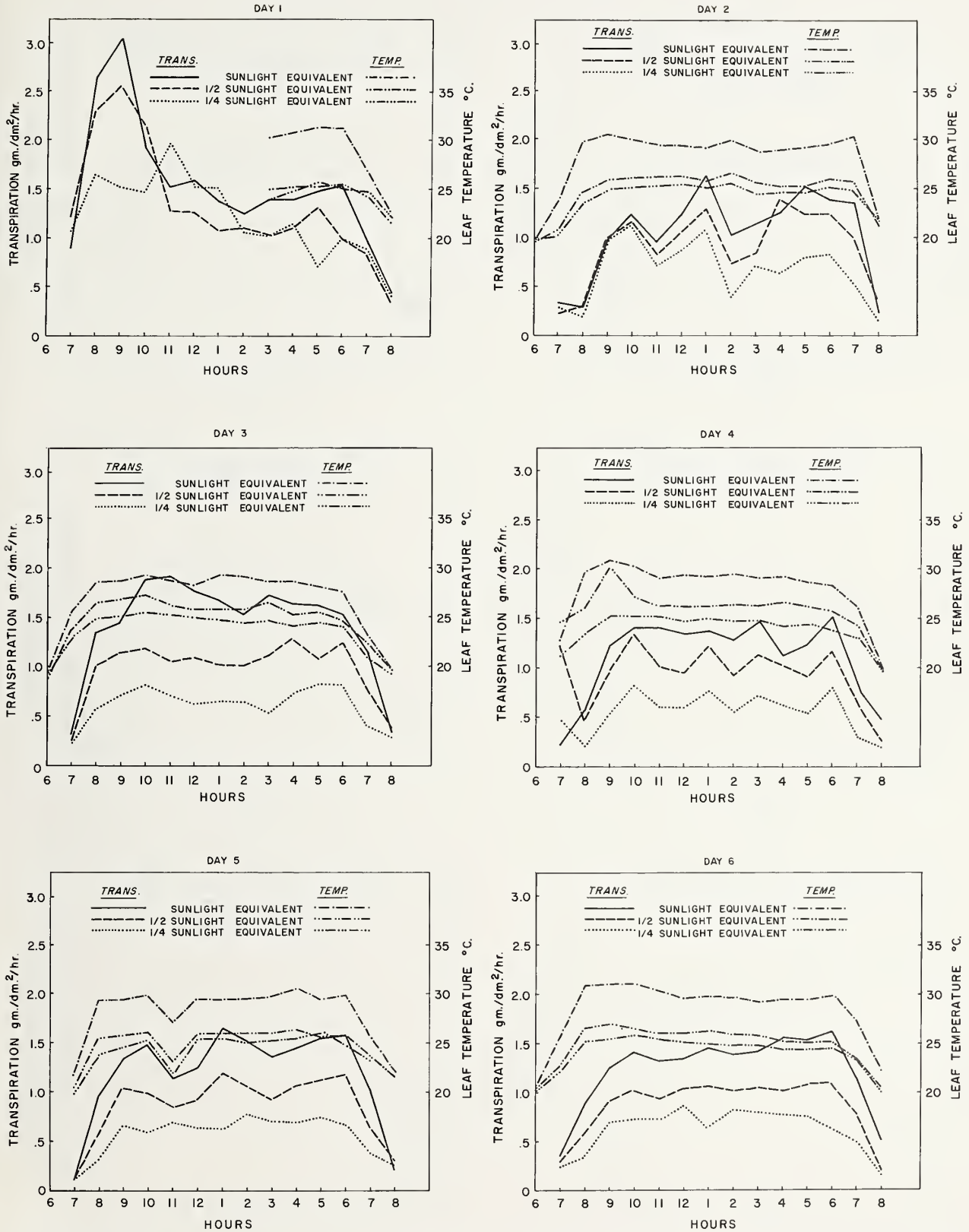

FIGURE 5.—Experiment 1 (days 1–6). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent.

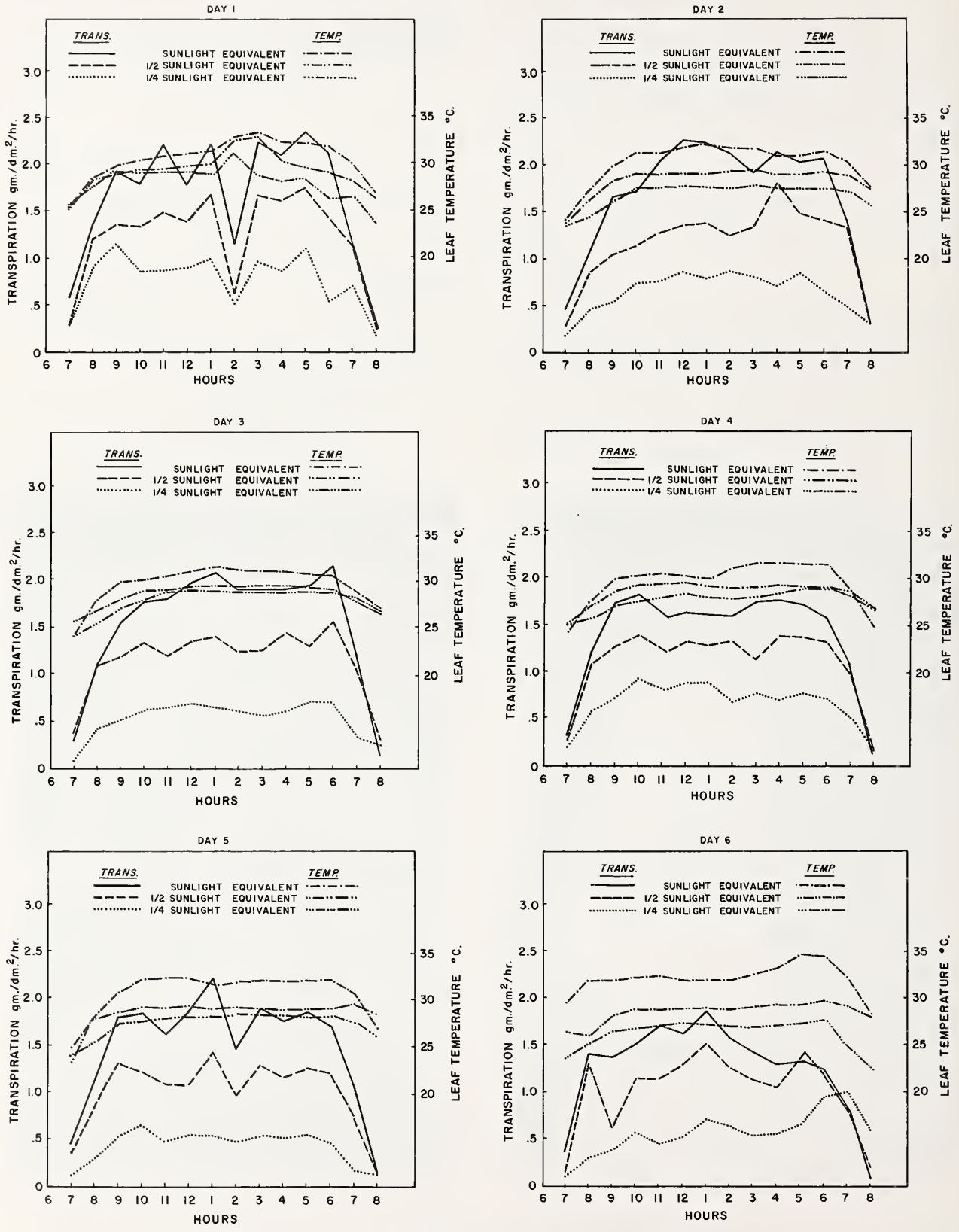

FIGURE 6.—Experiment 2 (days 1–6). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent; Krilium-treated soil.

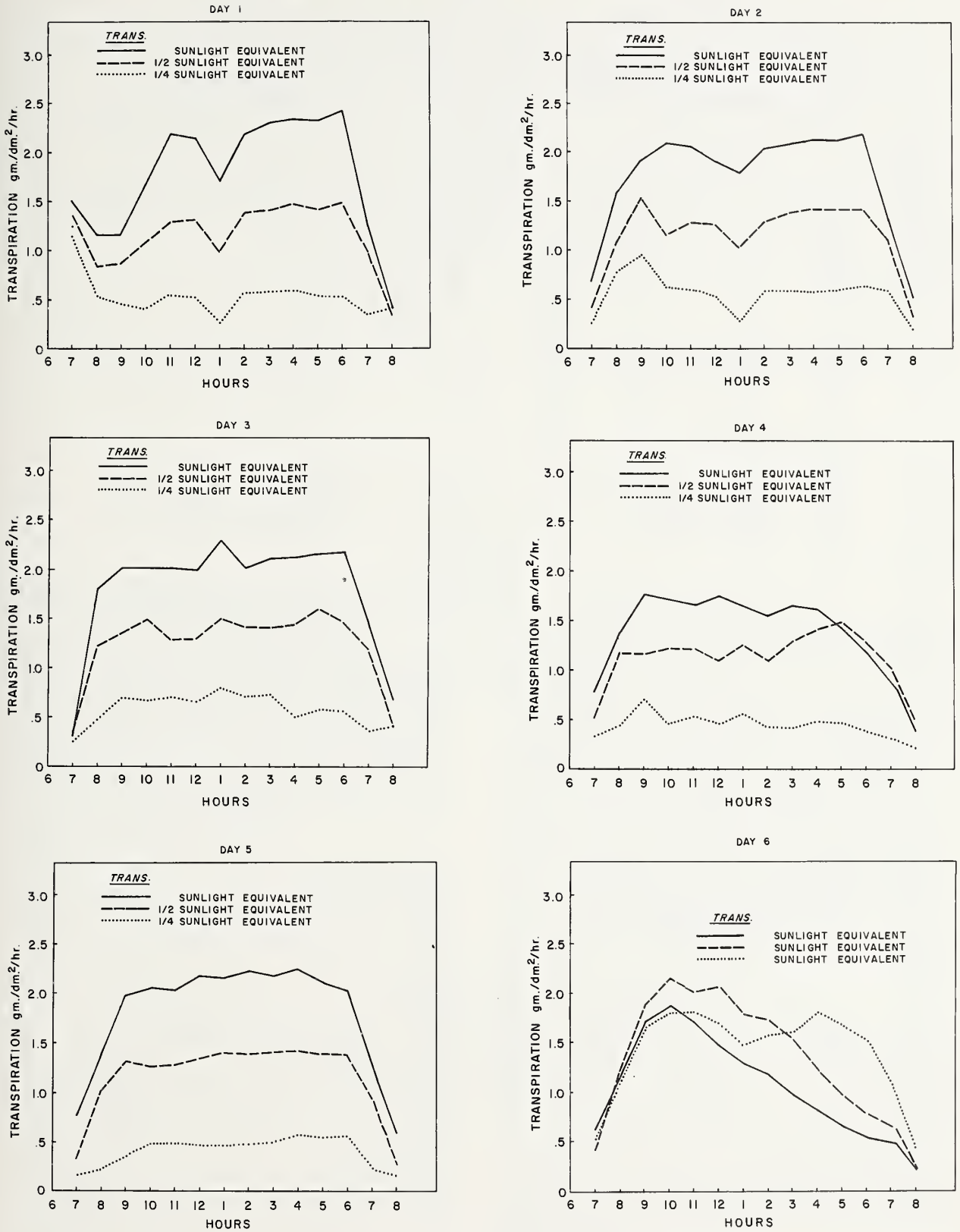

FIGURE 7.—Experiment 3 (days 1–6). Rate of transpiration of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent; Krilium-treated soil.

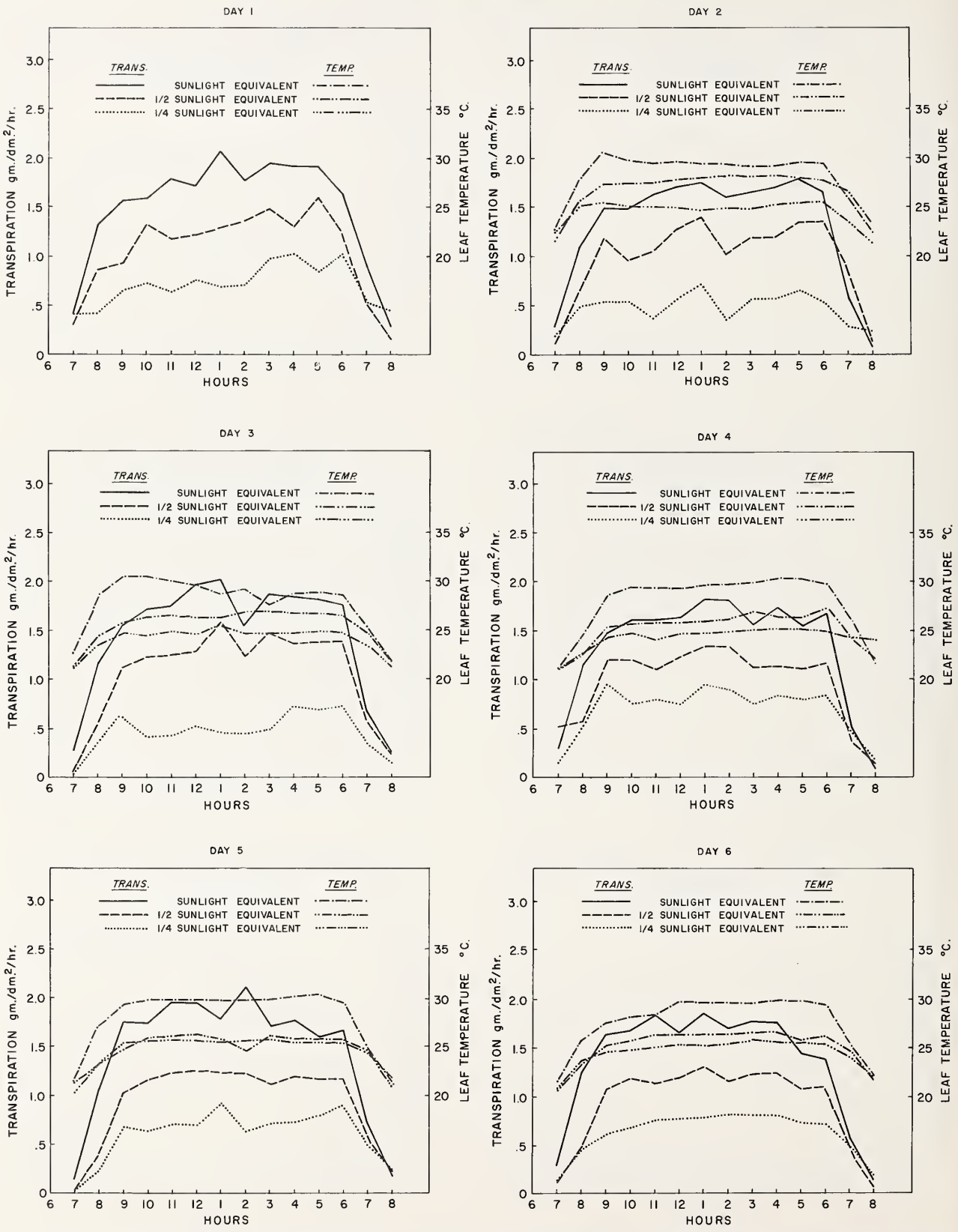

FIGURE 8.—Experiment 4 (days 1–6). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent; Krilium-treated soil.

TABLE 6.—*Average soil moisture tension during preconditioning part of experiments*

Experiment No.	Day	Soil moisture tension at indicated sunlight equivalent [1] from—					
		6 to 7 a.m.			7 to 8 p.m.		
		¼	½	Full	¼	½	Full
		Atm.	*Atm.*	*Atm.*	*Atm.*	*Atm.*	*Atm.*
2	1	0.08	0.08	0.08	0.10	0.13	0.16
	2	.12	.15	.17	.17	.27	.73
	3	.07	.07	.07	.09	.13	.19
	4	.09	.15	.21	.18	.48	2.17
	5	.07	.07	.07	.11	.19	.31
	6	.12	.21	.37	.27	1.9	4.5
3	1	.08	.08	.08	.09	.13	.17
	2	.11	.16	.20	.16	.37	1.22
	3	.07	.07	.07	.10	.15	.26
	4	.12	.18	.37	.18	.75	3.72
	5	.08	.08	.09	.13	.27	.94
	6	[2] .16	[2] .38	1.40	[2] 3.1	[2] 7.1	8.20
4	1	.07	.07	.07	.08	.09	.11
	2	.09	.10	.12	.10	.15	.22
	3	.06	.06	.06	.08	.09	.12
	4	.09	.10	.13	.14	.18	.33
	5	.07	.07	.07	.10	.15	.18
	6	.11	.16	.20	.21	.60	1.50

[1] ¼, ½, and full sunlight equaled 0.19, 0.52, and 0.85 cal. cm.$^{-2}$ min.$^{-1}$, respectively.
[2] Received full sunlight because of error in programing.

justed for leaf area. A highly significant covariate indicated any observed differences in transpiration per plant were mainly due to differences in leaf area. An investigation of the pairwise correlation coefficients between 39 variables (table 13 in appendix) indicates that $R_h \cdot L$, $V \cdot L$, $1nB \cdot L$, and $V \cdot B \cdot L$ are mainly responsible for variations in E_h. The correlation coefficients of $R_h \cdot L \times E_h$, $V \cdot L$, $1nB \cdot L$, and $V \cdot B \cdot L$ were 0.8913, 0.4732, 0.5980, and 0.3207, respectively.

Starting with day 8 and continuing thereafter, as available water decreased and soil moisture tension (as measured by matric suction) increased, transpiration decreased (figs. 9–12). Transpiration on day 8 at high vapor pressure deficits (v.p.d.) (30-percent relative humidity) (fig. 12) was at least twice that at low v.p.d. (90-percent relative humidity) (fig. 10). At intermediate v.p.d. (fig. 11) transpiration was also intermediate. The inability of the plant to meet the evaporative demand was demonstrated on day 9 at high v.p.d. under both high and medium light (fig. 12), whereas only under high light was transpiration continually reduced at intermediate v.p.d. on day 9 (fig. 11). At low v.p.d. no comparable transpiration drop could be seen. The reductions in transpiration were due to an inability of water transport from soil through the plant to meet evaporative demand.

Radiant energy, v.p.d. of the air, and increasing soil moisture tension have a rather marked effect on transpiration (figs. 13–16).

Analysis of the first drawdown cycle in each experiment gives the following conclusions: Experiment 1 was comparable with experiment 3 regarding environmental factors imposed (air v.p.d. of 12.6 mm. Hg.). In experiment 1 Krilium-treated soil was not utilized. The poor aggregation evidently affected the availability of water for transpiration as well as growth (plant growth quadrupled in Krilium-treated soil). Thus soil moisture tension limited transpiration near 0.1 atmosphere in experiment 1 (fig. 13) at both high and medium light intensities, whereas in experiment 3 (fig. 15) at all three light intensities soil moisture availability limited transpiration near 1 atmosphere.

In experiment 2 (fig. 14), where air v.p.d. was equal to 3.2 mm. Hg., at full light intensity transpiration dropped off because of limiting soil moisture near 1 atmosphere, at medium light it dropped near 2 atmospheres, whereas at low light the change in slope of the curve was not distinct.

In experiment 4 (fig. 16) at an air v.p.d. of 22.2 mm. Hg., soil moisture tension limited transpiration near 1 atmosphere at the lower light intensities, but near 0.1 to 0.2 atmosphere it already appeared to be limiting transpiration at the high light intensity.

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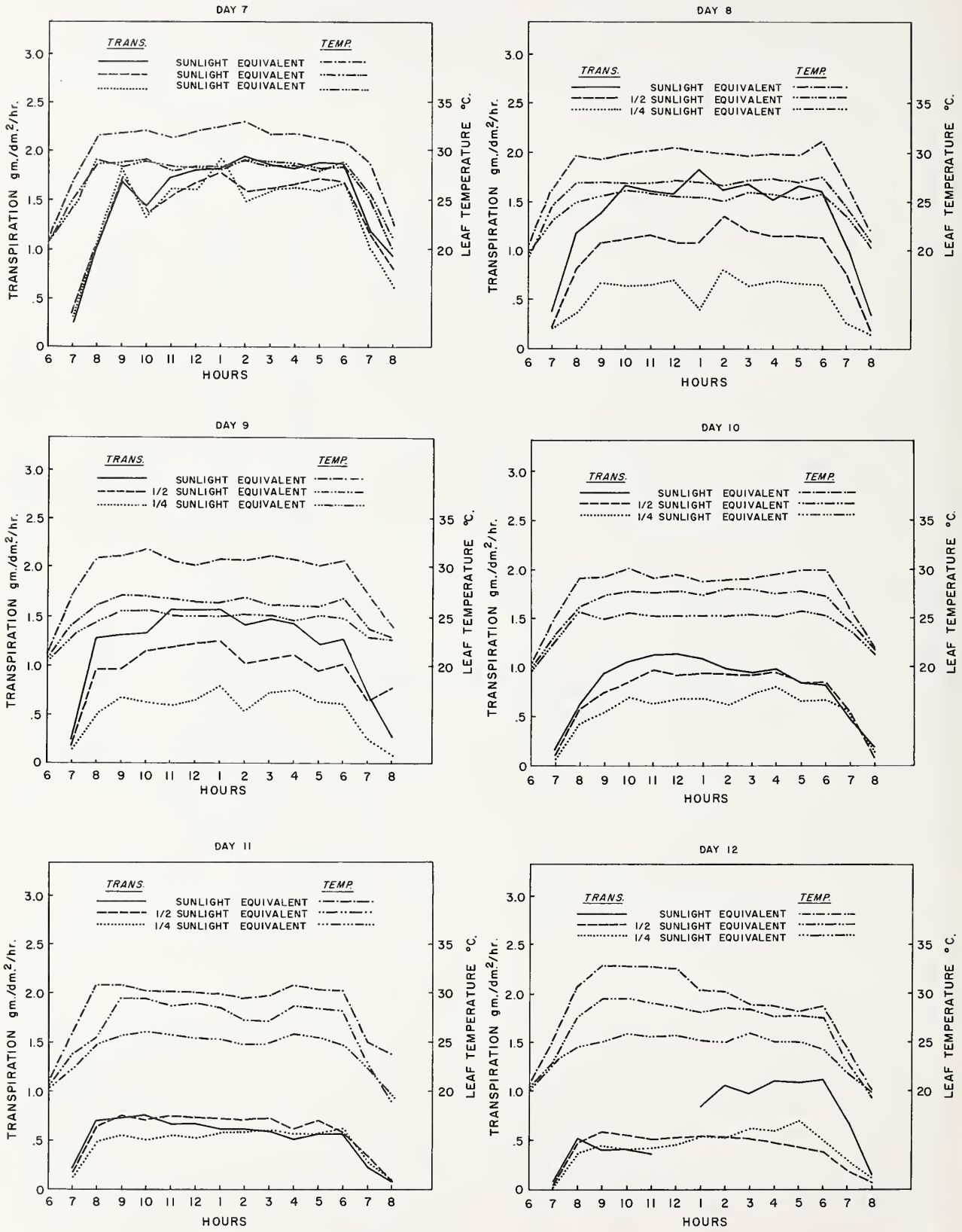

FIGURE 9.—Experiment 1 (days 7–16). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent.

Since the effects of low moisture tension on root vitality and moisture uptake are unknown, the second drawdown cycles are not discussed, but are presented in figures 13 through 16. During wilting, changes occur in the root system, which reduce the water absorbing or water conducting capacity of the roots (*11*, *12*). Complete interpretation must await further experimentation. These findings on moisture tension effects agree remarkably well with the studies of Gardner and Ehlig (*28*).

The effect of radiation level on leaf temperature is very pronounced (figs. 5, 6, and 8). At one-fourth sunlight equivalent the average temperature of the upper and lower surface of the leaf was nearest ambient air temperature (25° C.); at one-half sunlight equivalent it was as much as 4° above ambient temperature, and at full sunlight equivalent at least 5° above ambient temperature. Ansari and Loomis (*2*) also found a high correlation between radiant energy and leaf temperature. In general, throughout these studies the temperature of the upper leaf surface was higher than that of the lower surface.

In the soil moisture tension studies, leaf temperature was affected by the rate of transpiration. In experiment 4 on day 8 (fig. 12) all leaf temperatures are lower than those on the comparable day at lower v.p.d. in experiments 2 and 3 (figs. 10 and 11). A depression of the leaf temperature by transpiration under high light intensities occurred after irrigation on day 11 in experiments 2–4 (figs. 10–12) and on day 12 in experiment 1 (fig. 9). Leaf temperature also dropped under medium light after irrigation on day 12 in experiments 3 and 4 (figs. 11 and 12). Under low light a perceptible drop in leaf temperature after irrigation is apparent only on day 13 in experiment 4 (fig. 12) at the highest air v.p.d. used in these studies.

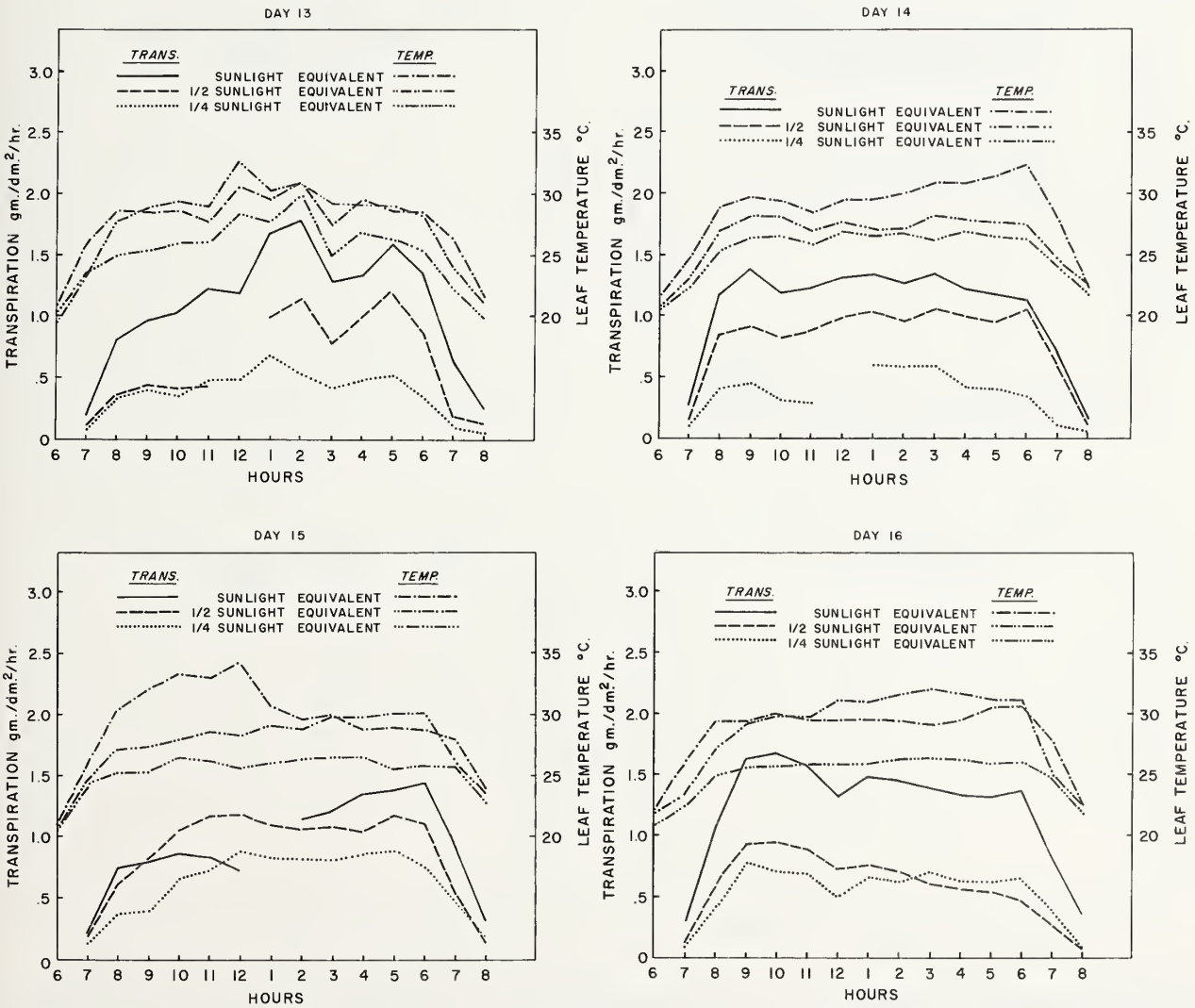

FIGURE 9.—Experiment 1 (days 7–16). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent—Continued.

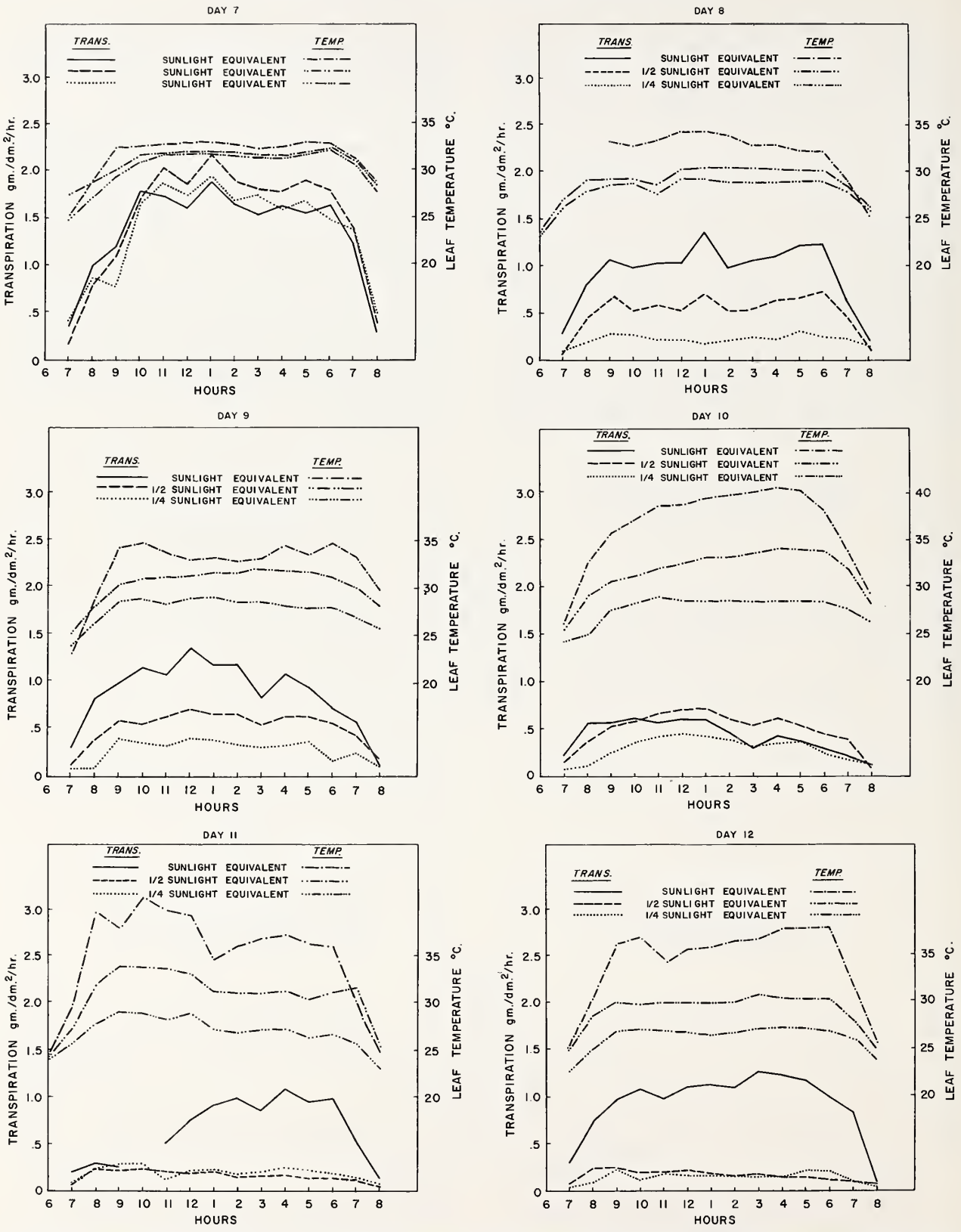

FIGURE 10.—Experiment 2 (days 7–17). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 90 percent; Krilium-treated soil.

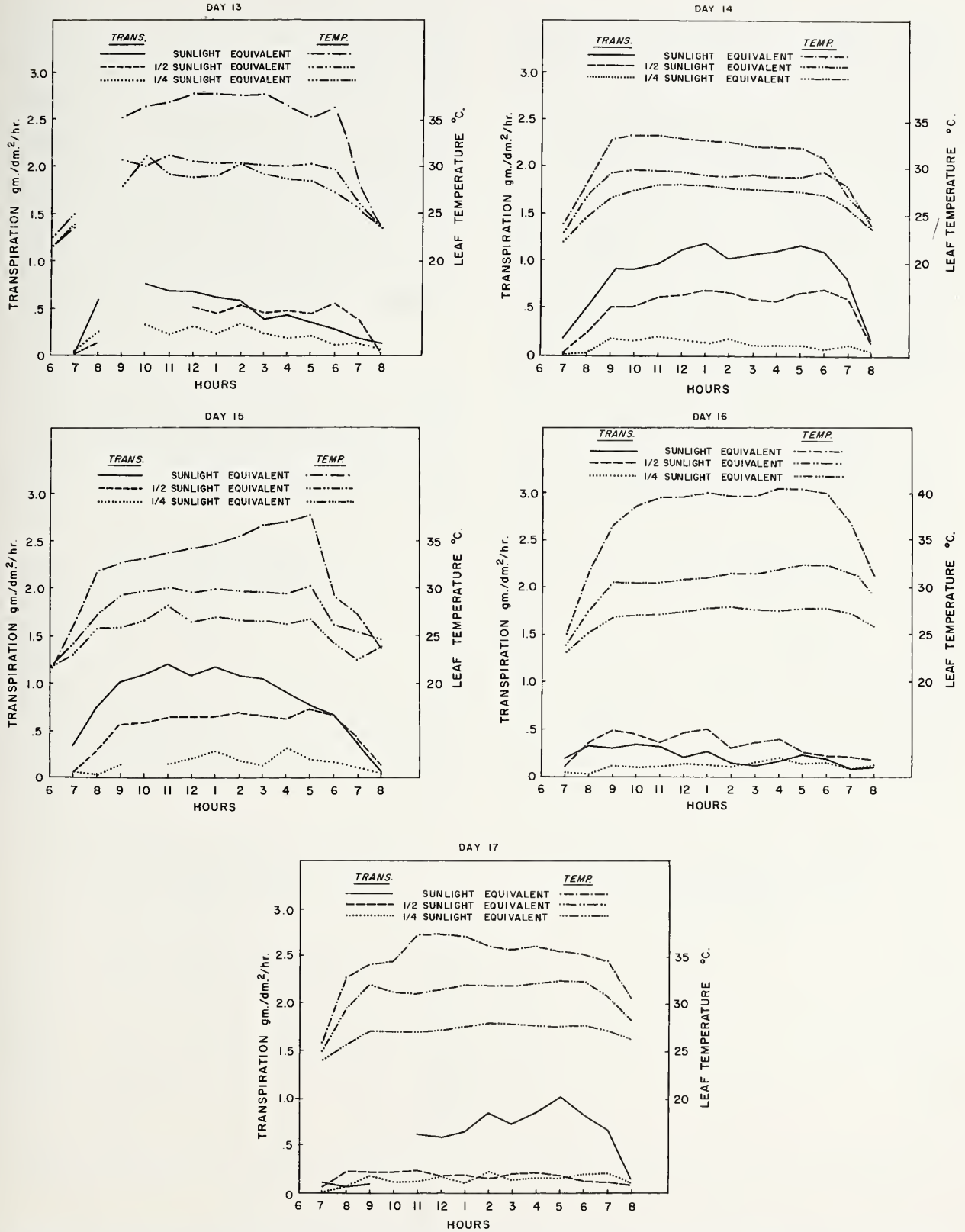

FIGURE 10.—Experiment 2 (days 7–17). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 90 percent; Krilium-treated soil—Continued.

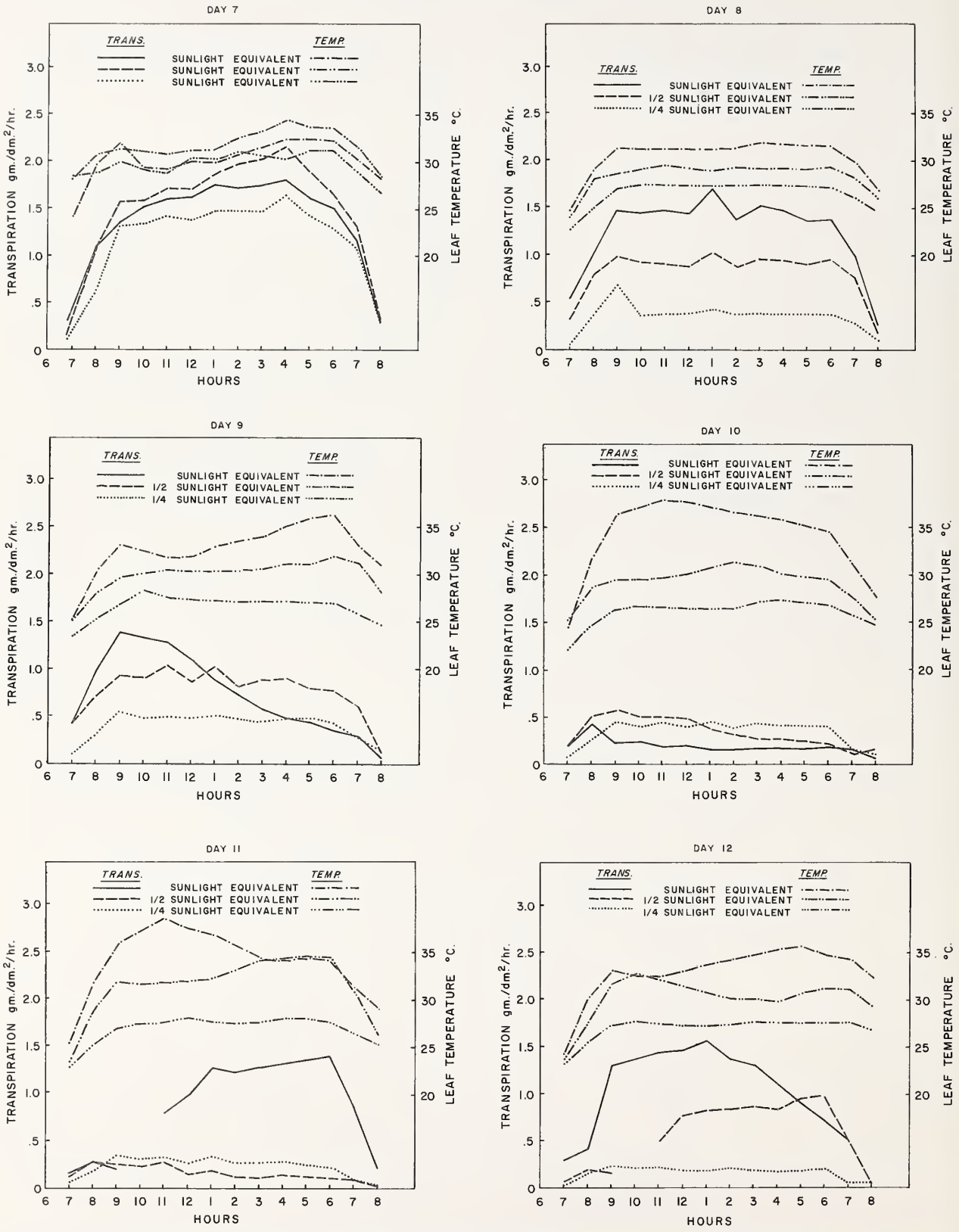

FIGURE 11.—Experiment 3 (days 7–15). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent; Krilium-treated soil.

These data show that transpiration can lower leaf temperature, but its ability to do so is markedly affected by the amount of radiant energy, soil moisture availability, and air v.p.d. That transpiration cools leaves is a generally accepted phenomenon; however, how much and whether below air temperature seem to depend on the species and its environment. This is evidenced in the review by Clum (*15*) as well as in his work (*16*) and the work of others (*2*, *14*, *24*, *43*, and *73*). The failure to recognize interactions between radiant energy, soil moisture tension, and air v.p.d. have led to apparent discrepancies in the literature as to the virtue of transpiration as a factor of importance to the plant (*19*).

Stomatal Activity

Stomatal activity is summarized in figures 17–20. The percentages of open stomata in figure 17 are now known to be too large. In experiment 1 an attempt was made to determine when the guard cells were beginning to part; however, it was found that detection of this stage is subject to considerable error in judgment. The inability to quantify this stage was discussed by Stalfelt (*63*), and his conclusion is correct that no method exists by which a hermetic stomatal closing can be directly determined. After experiment 1 if a stoma did not allow light to pass through the pore, it was considered closed. These criteria apply to the data reported in figures 18–20.

Under the previously described growing conditions, the corn stomata were mostly inoperative until 13 days after sowing. Figures 17–20 indicate that stomatal operation was generally greatest during morning hours throughout the preconditioning part of the experiment. Under any condition, considerably more of the stomata opened on the lower epidermis than on the upper epidermis. In general, more stomata were opened under full than under one-half sunlight equivalent, and more were opened under one-half than one-fourth sunlight equivalent when soil moisture tension was not affecting stomatal operation.

The relatively high stomatal activity on day 7 (figs. 18–20), the first day after the preconditioning study, suggests that the sequence of previous

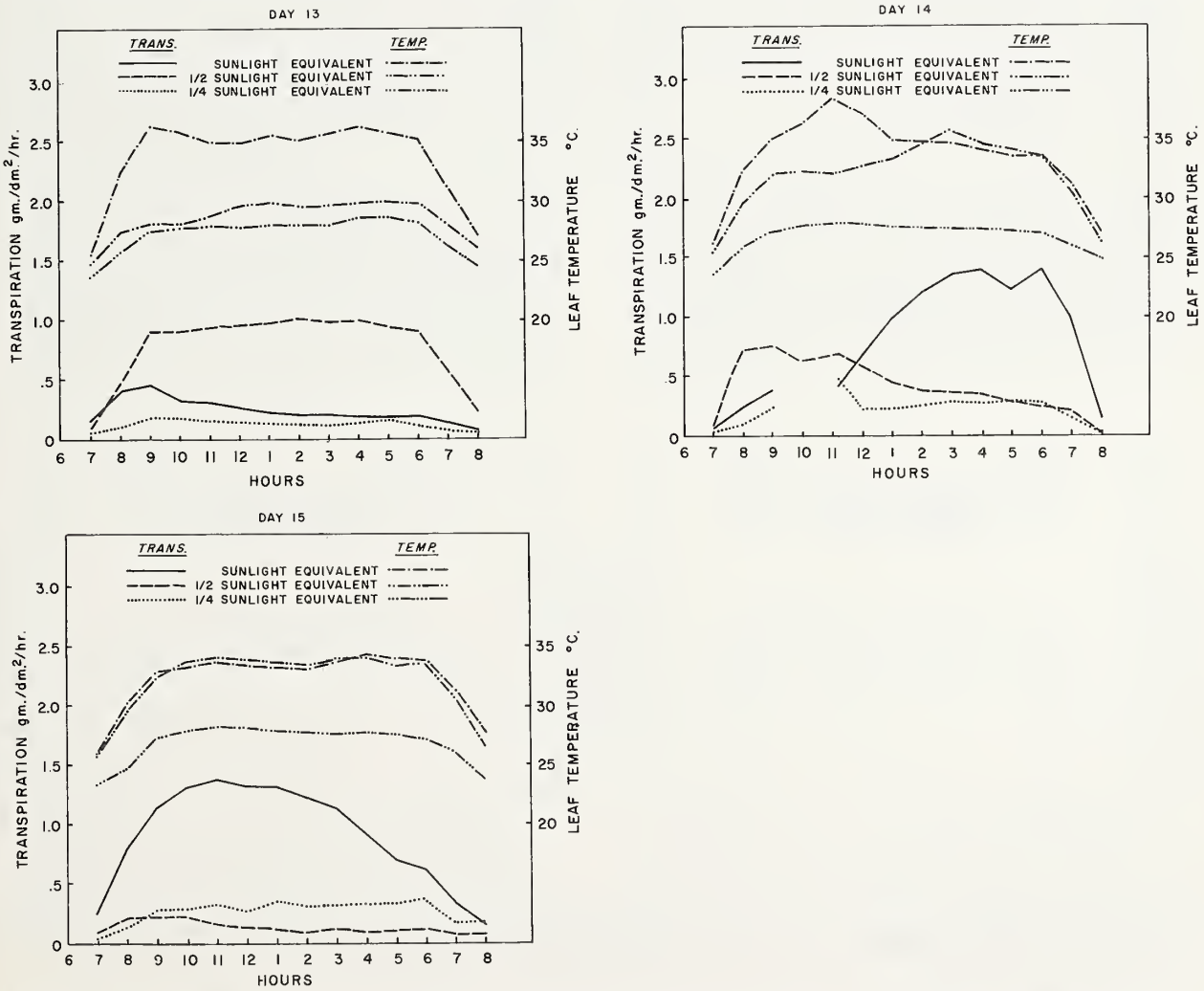

FIGURE 11.—Experiment 3 (days 7–15). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 60 percent; Krilium-treated soil—Continued.

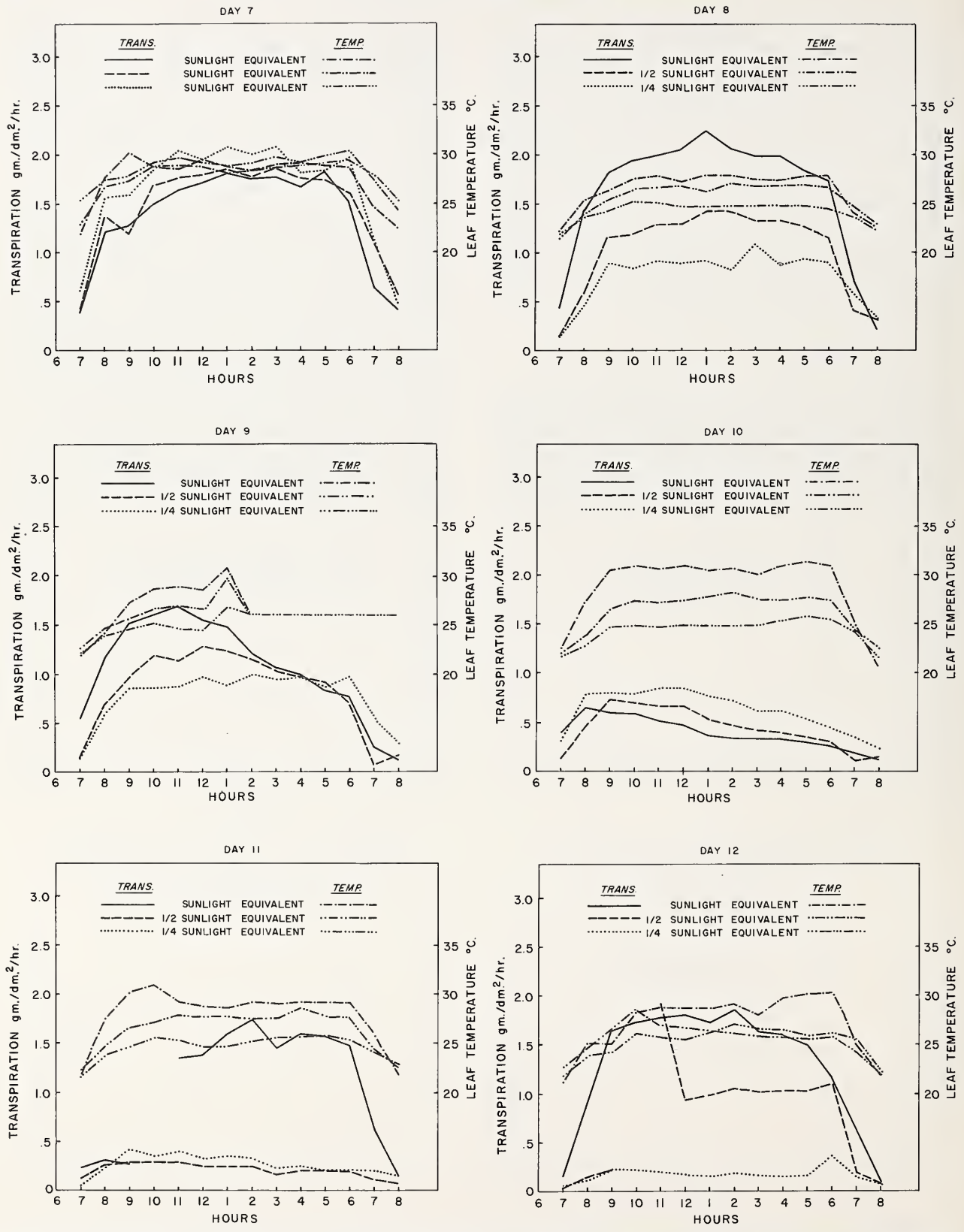

FIGURE 12.—Experiment 4 (days 7–14). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 30 percent; Krilium-treated soil.

conditioning for group A (table 5) was most conducive to stomatal opening on day 7. Analysis of variance (table 14 in appendix) of the differences in percentage stomata open (lower epidermis) was carried out after transforming the percentages into angle by arc sine formula. The data included percentages for 10 periods (periods 3–12) for each bay. The analysis indicated that there was a highly significant difference between the bays but not the periods. Duncan's multiple-range test showed that stomatal response in bay 2 was significantly different from that in bays 1 and 3, but bays 1 and 3 did not differ between themselves. In other words, the sequence of light from high-low-medium established a condition for higher subsequent stomatal activity than did the low-medium-high or medium-high-low sequences. This is logical if one considers the hydroactive and photoactive effects already discussed (*51*).

Guard cell operation during the soil moisture tension studies was measurably affected by moisture availability. This is shown by the decrease in activity as soil moisture tension increased (figs. 18 and 19). Irrigation occurred around 10 a.m.; however, only in figure 17 is shown a resumption of activity on the day of irrigation. These findings are contrary to Iljin's postulation (*33*) that an abnormally high number of stomata would open immediately in response to irrigation and release of water deficit. Polster and Fuchs (*54*) presented evidence that guard cell activity is impaired during a period of drought.

The importance of the hydroactive phase in guard cell operation is indicated in the results. Decreases in the free energy of water in the soil or the atmosphere could upset what may be a delicate balance of the plant with its environment. Internal water balance is controlled by the relative rates of water absorption and water loss (*36*). Changes in water balance were indicated by the differences in the water content of plants (percentage by weight) at the start of soil moisture drawdown, just prior to irrigation, and again several days after irrigation. These changes in plant moisture content under wet and dry soil conditions may be seen in the data in table 7.

On the average, only a few percent changes in total plant water were realized during the drying-rewatering cycle in any of the studies. A change of the same order of magnitude was measured by relative turgidity. This small change is understandable when one considers that only the water of the nonliving system, such as intercellular spaces, the xylem, and the cell wall matrix, is readily available for evaporation. To remove, in the transpiration process, bound water of this nonliving system and water of the living system (protoplasm and its inclusions) would require a tremendously higher energy input. If much of the water from the living cells is removed, death of the plant ensues. The resistance of protoplasm to desiccation is probably very important in drought resistance and needs much further study.

These studies indicate that changes in the resistance to the flow of water in the plant do not necessarily involve visible stomatal action, as demonstrated in the lack of open stomata (fig. 20) in the soil moisture part of experiment 4, where decided soil moisture tension effects were found (fig. 16).

Photosynthetic Rate

Figures 21 and 22 show that as soil moisture tension increases, photosynthesis drops to the compensation point. Daily photosynthesis curves at full sunlight resemble comparable daily transpiration curves and are similar to Brix's (*12*) findings with tomato and loblolly pine plants. The changes of either are attributable to soil moisture tension effects.

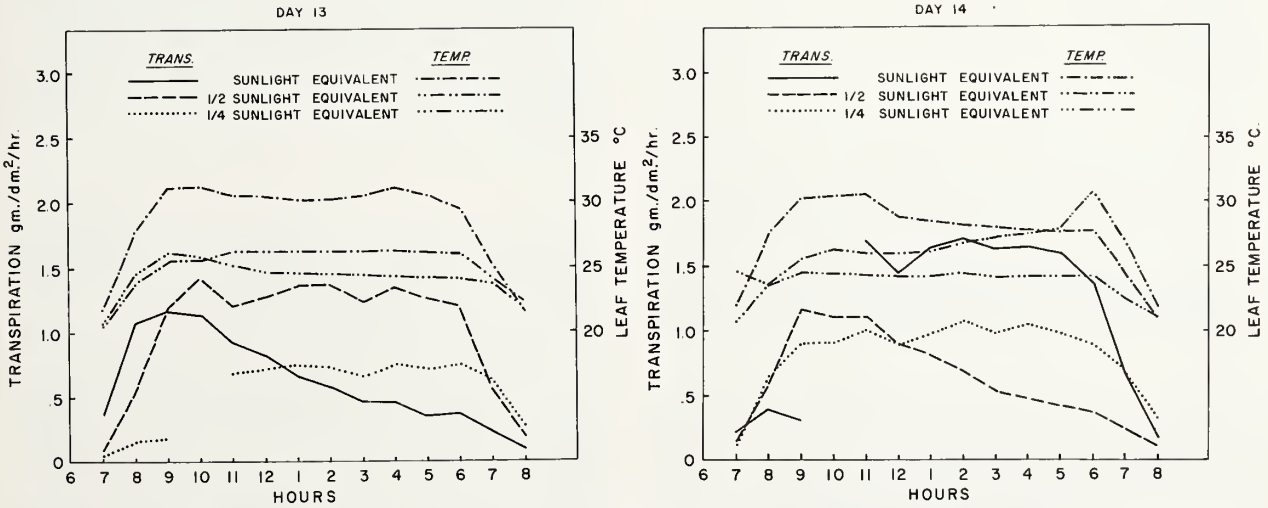

FIGURE 12.—Experiment 4 (days 7–14). Rate of transpiration and leaf temperature of corn plants grown at indicated light intensities; ambient air temperature 25° C., relative humidity 30 percent; Krilium-treated soil—Continued.

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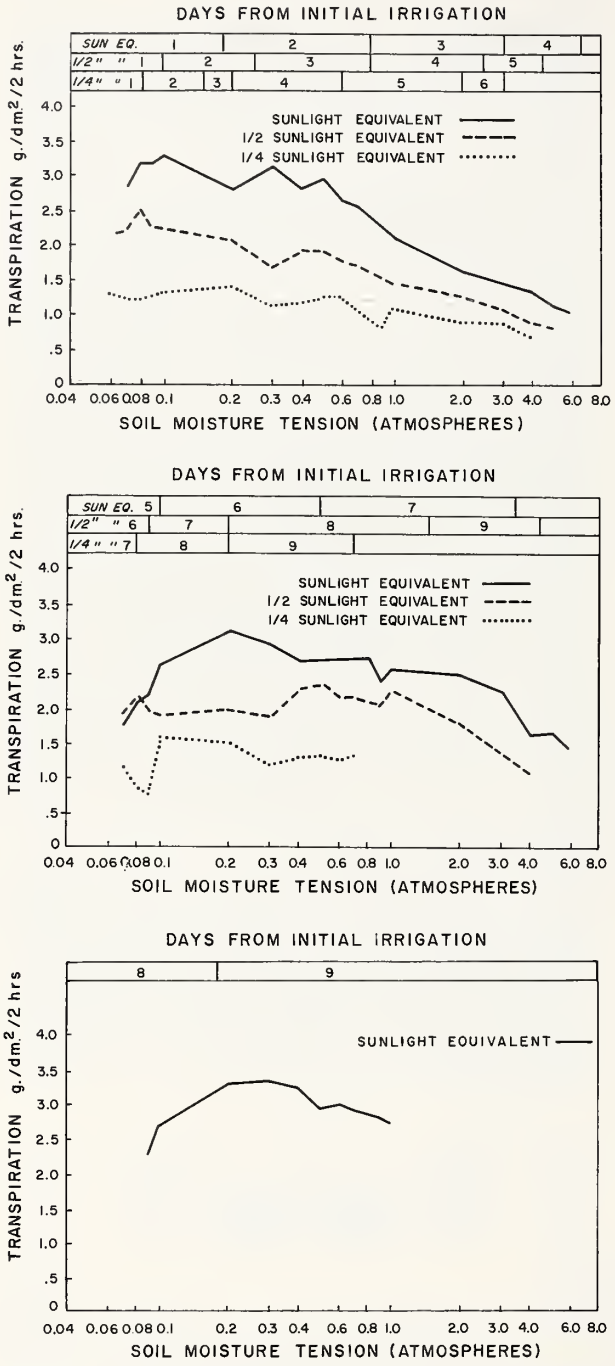

FIGURE 13.—Experiment 1. Rate of transpiration at indicated light intensities as affected by soil moisture tension during irrigation-drying-reirrigation cycles; ambient air temperature 25° C., relative humidity 60 percent.

Beginning on day 10 the soil moisture tension was less than 6 atmospheres; however, apparent photosynthesis had already been reduced to a minimum. These findings complement the work of Denmead and Shaw (*21*) with regard to the effects of extended soil moisture tension on transpiration and corn yield. These findings are also in agreement with Brix (*12*), Ashton (*5*), Zholkevich et al. (*82*), McCune,[3] and Kramer (*38*) concerning soil water availability and photosynthesis. Most plants show a decrease in production when moisture stress exceeds 1 atmosphere (*29*).

Low radiant energy resulted in low moisture tension effects on photosynthesis in these studies, as indicated in the graphs. At the low light intensities our data with corn are comparable to data obtained by Upchurch et al. (*71*) showing little soil moisture tension effect on clover photosynthesis. Upchurch worked at light intensity levels below photosynthetic saturation as indicated by Beinhart's (*6*) work; therefore, his data certainly do not hold under all conditions in the field since his radiant energy was limiting.

Figures 21 and 22 indicate an apparent correlation of photosynthesis with carbon dioxide (CO_2) concentration on day 8. Soil moisture tension lowered photosynthesis by day 9 and increasing tensions through the day appear to be constantly lowering photosynthesis, although CO_2 concentration of the room affected the magnitude of the photosynthetic rate. This is difficult to reconcile if one considers only one factor to be limiting at any one time. Increased photosynthesis with increases in CO_2 concentration at a constant light intensity is also reported by Hesketh and Moss (*32*) as well as by Gaastra (*26*).

Even more difficult to understand is the indication of a limiting effect by CO_2 concentration (fig. 22) on days 12 and 13 at one-half and one-fourth light intensity. Since a measurable drop in overall photosynthesis can be seen from full light to lower light intensities, one would expect light to be the limiting factor, at least at the lower light intensities. Much additional information is needed to reconcile these results; however, the effect noted is most probably related to changes in the diffusional resistance to CO_2 uptake by the leaf, as discussed by Brix (*12*). Since the stomata appeared closed, the diffusional resistance must have involved CO_2 passage through the epidermis. It is striking, however, that such high rates of photosynthesis could be maintained by near-cuticular diffusion.

Transpiration and Guard Cell Operation of Two Grain Sorghums and a Grain Sorghum-Sudangrass Cross

In a field study with several grain sorghum hybrids and varieties, it was observed that certain strains showed signs of soil moisture stress, i.e., rolling of the leaves, several days before other

[3] MCCUNE, D. L. EFFECT OF MOISTURE TENSION ON THE PRODUCTION AND MOVEMENT OF CARBOHYDRATES IN PLANTS. 1957. [Unpublished Ph. D. dissertation; on file at Purdue Univ., Lafayette, Ind.]

TABLE 7.—*Water in corn plants with adequate and insufficient soil moisture to meet evaporative demands*

Experiment No.	Day	Sunlight equivalent	Water in corn plants when soil moisture tension is—		Relative turgidity
			0.05 atmosphere	8–15 atmospheres	
			Percent	*Percent*	*Percent*
2	7	Full [1]	91		
	11	do		81	83
	13	½		81	89
	15	¼		87	95
	17	Full	87		
	17	½	88		
	17	¼	90		
3	7	Full [1]	89		
	11	do		86	
	12	½		85	
	14	¼		86	
	15	Full	86		
	15	½	87		
	15	¼	89		
4	7	Full [1]	89		
	11	do		86	
	12	½		87	
	13	¼		87	
	14	Full	89		
	14	½	89		
	14	¼	90		

[1] Representative of all plants used in the drawdown cycle.

strains. It was thought that this variation in time of showing moisture stress was probably due to differences in transpiration rates by the various strains of sorghum, and that the different transpiration rates might be related to stomatal behavior. Therefore, an experiment was designed to evaluate transpiration and stomatal behavior of two grain sorghum varieties (Martin and CK–60) and a grain sorghum-sudangrass cross (DeKalb SX–11) under several light, humidity, and soil moisture conditions. The study also provided needed information regarding the effect of a plant's environment on transpiration and stomatal behavior.

Miller (*42*) concluded that light may increase transpiration of plants by raising the leaf temperature, by imparting kinetic energy to molecules of water within the leaf, or by increasing through physiological processes the permeability of protoplasm and cell walls to water. On the other hand, Kramer (*37*) stated that light greatly modified transpiration through its effects on stomatal opening. Slatyer (*61*) considered transpiration to be a passive process, with its rate determined primarily by the vapor pressure gradient between the leaf and the outside air. Kramer (*37*) agreed that the rate of transpiration, barring stomatal control, was dependent on the steepness of the vapor pressure gradient from plant tissue to outside air.

Other workers (*3*, *7*, *10*) have shown reductions in transpiration as relative humidity was increased. Lloyd (*39*) and other workers (*3*, *20*, *30*, *40*) have shown that the stomata of various plants opened when exposed to light, and it is generally concluded (*31*, *40*, *77*) that plant stomata open more readily at higher humidities.

Gray and Peirce (*30*), by making direct microscopic examination of wheat stomata, discovered that stomatal activity was reduced by limited soil moisture.

Schneider and Childers (*58*) found that apple stomata closed when the plant was allowed to wilt and that the stomata reopened within 3 hours after watering. Furr and Degman (*25*), also working with apples, observed a marked decrease in stomatal opening due to increased soil moisture tension, even when soil moisture content was several percent above permanent wilting percentage. Work with peaches (*18*) has also shown that low soil moisture reduced stomatal activity.

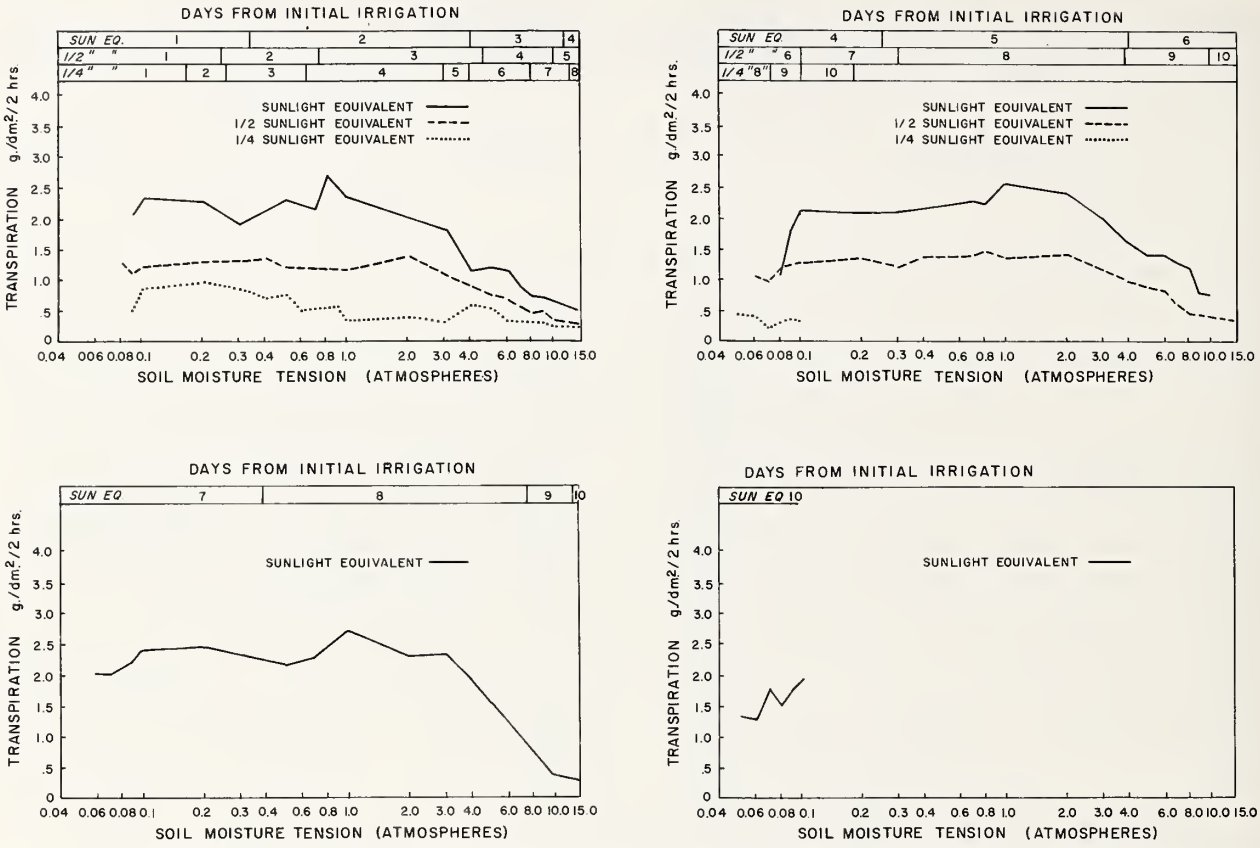

FIGURE 14.—Experiment 2. Rate of transpiration at indicated light intensities as affected by soil moisture tension during irrigation-drying-reirrigation cycles; ambient air temperature 25° C., relative humidity 90 percent; Krilium-treated soil.

In contrast to these findings regarding the effect of soil moisture on stomatal behavior, Veihmeyer and Hendrickson (*72*) reported that no differences could be detected in stomatal opening of fruit trees growing in relatively dry or saturated soils when the moisture content was above the wilting coefficient.

Procedure

The experimental procedure for this study has previously been reported (*51*) except for the procedure used to evaluate stomatal behavior. However, for purposes of discussion, the treatments used in the light and humidity study are repeated in table 8.

Hourly stomatal observations were made each day by means of a "stomata camera" (*75*). Observations were made on two plants of each strain in each of the three replications, or on a total of 18 plants. The third leaf from the top of the plant was selected for stomatal studies, since this was usually the most mature leaf that was not shaded by other leaves on the same plant. Two fields—near the middle of the leaf and on opposite sides of the midrib—were observed on each leaf.

TABLE 8.—*Schedule of light and humidity conditions*

Day of week	Light intensity [1]	Relative humidity
		Percent
Monday	½	60
Tuesday	Full	90
Wednesday	do	60
Thursday	do	30
Friday	½	90
Saturday	½	30
Sunday [2]	Full	60
Monday	do	30
Tuesday	½	90
Wednesday	Full	90
Thursday	½	60
Friday	Full	60
Saturday	½	30

[1] Full light and one-half light intensity equaled 1.35 and 0.65 cal. cm.$^{-2}$ min.$^{-1}$, respectively.
[2] No data collected.

A stomatal index system, which placed each stoma into one of four classes, depending on its apparent degree of opening, was used to evaluate

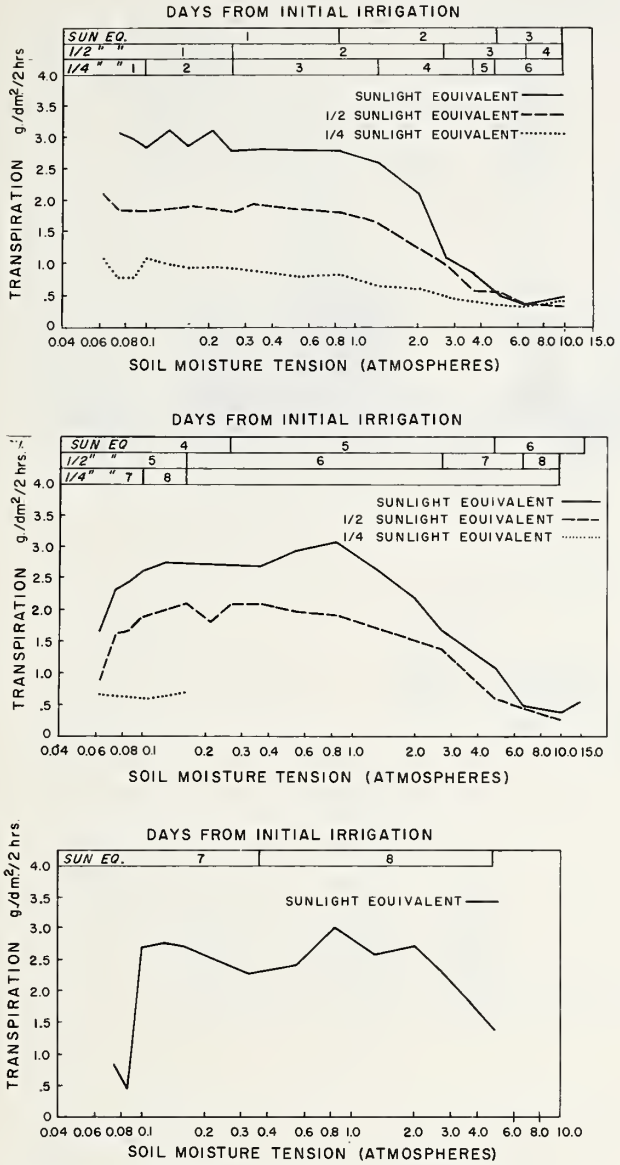

FIGURE 15.—Experiment 3. Rate of transpiration at indicated light intensities as affected by soil moisture tension during irrigation-drying-reirrigation cycles; ambient air temperature 25° C., relative humidity 60 percent; Krilium-treated soil.

stomatal behavior quantitatively. The classes of stomatal opening were as follows:

Class	*Degree of stomatal opening*
1	Closed.
2	Cracked open to ¼.
3	¼ to ¾.
4	Over ¾.

As each field was observed the number of stomata in each class was recorded. Then the percentages of stomata in each field in classes 2, 3, and 4 were multiplied by 1, 2, and 3, respectively. These values were then added to give the "stomatal index" for the field observed. The number of stomata in class 1 was used only to determine the total number of stomata in each field.

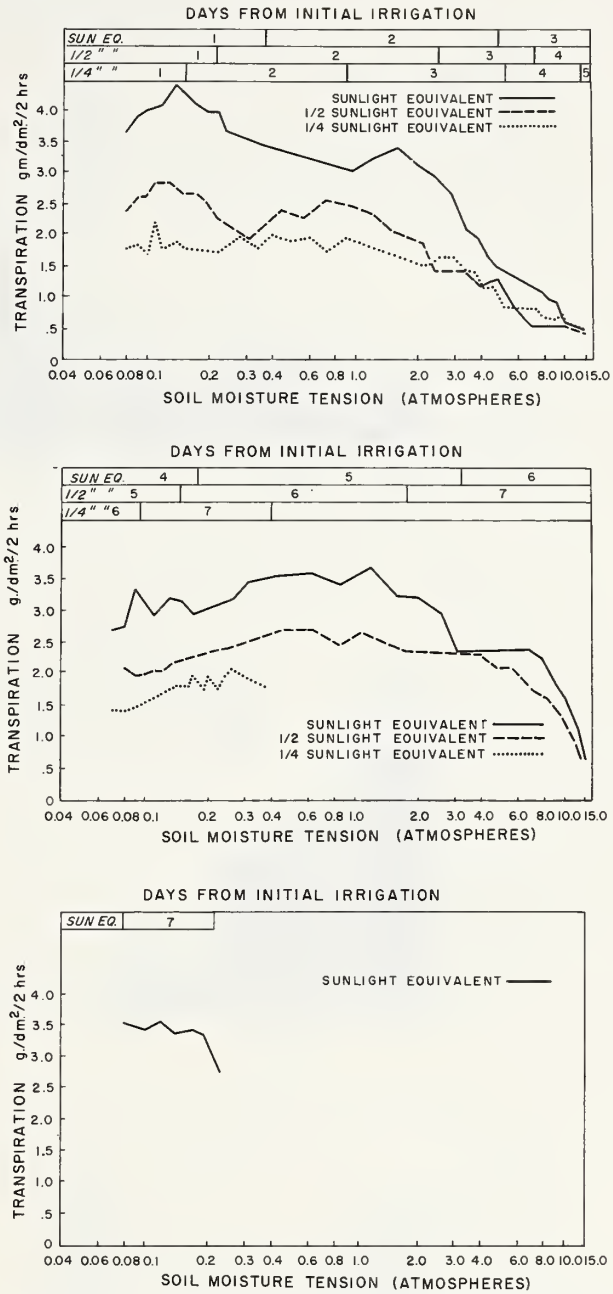

FIGURE 16.—Experiment 4. Rate of transpiration at indicated light intensities as affected by soil moisture tension during irrigation-drying-reirrigation cycles; ambient air temperature 25° C., relative humidity 30 percent; Krilium-treated soil.

Results and Discussion

Transpiration.—The bar graphs in figures 23 and 24 show the effect of two light levels and three relative humidities on daily transpiration by the

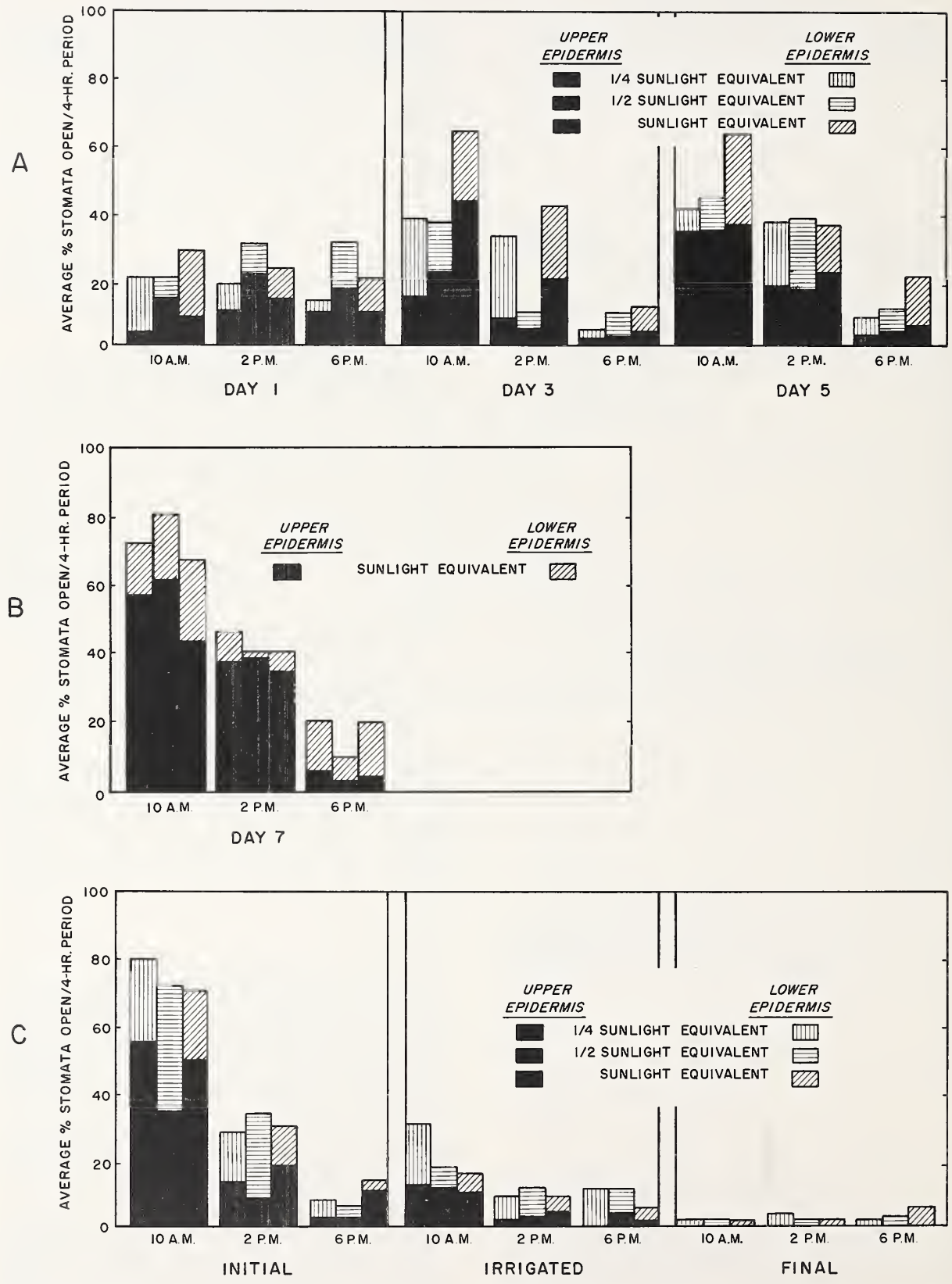

FIGURE 17.—Experiment 1. Percentage of stomata open on upper and lower epidermis of corn leaves at indicated light intensities. Individual bars are average of preceding 4 hours' activity. *A*, Preconditioning study; *B*, conditioning; *C*, soil moisture tension study: Initial (day 8); irrigated (day of reirrigation varies at each light intensity, see fig. 13); final (day 16), termination of experiment.

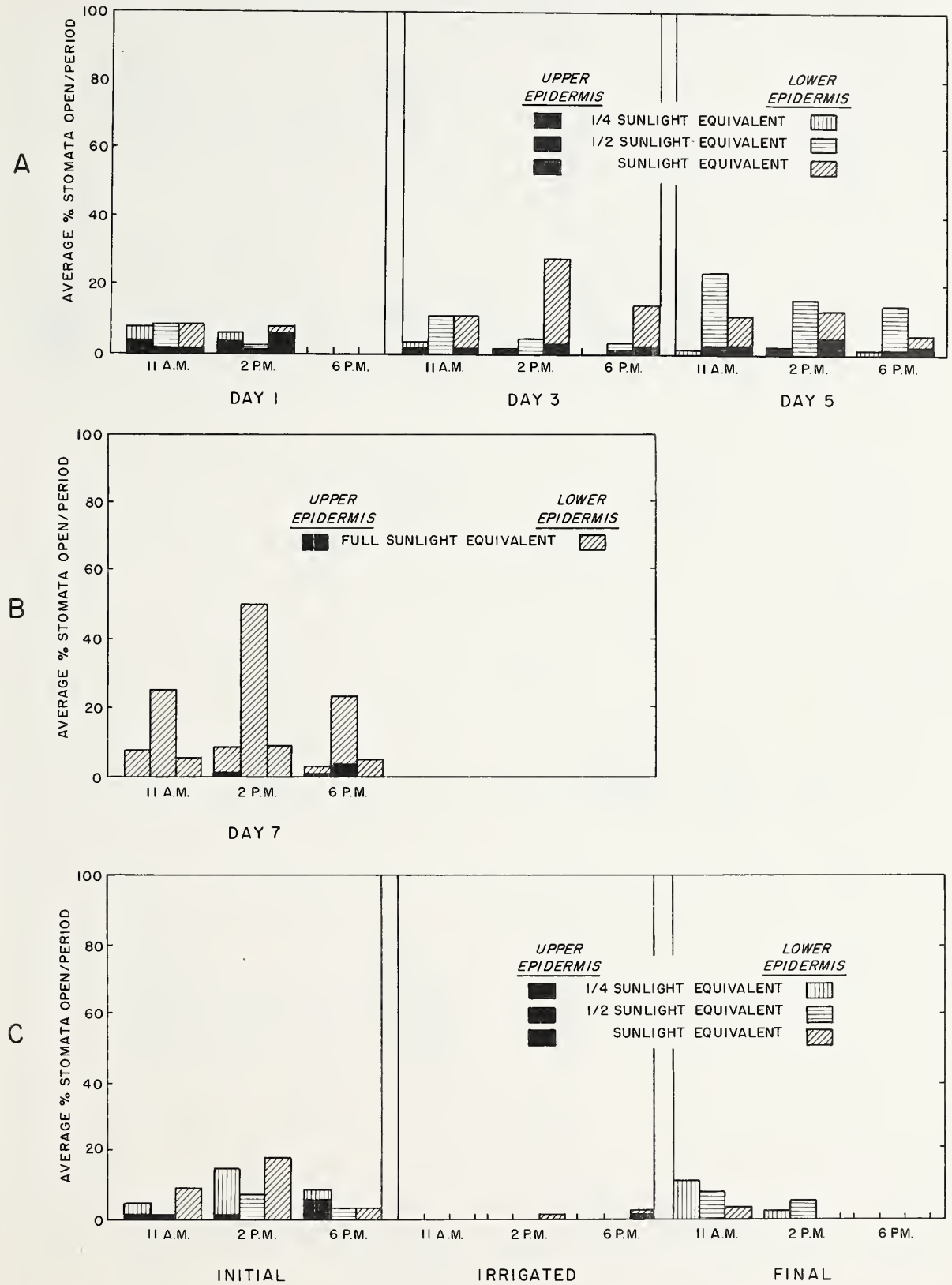

FIGURE 18.—Experiment 2. Percentage of stomata open on upper and lower epidermis of corn leaves at indicated light intensities. Individual bars are average of preceding 3 hours' activity. *A*, Preconditioning study; *B*, conditioning; *C*, soil moisture tension study: Initial (day 8); irrigated (day of reirrigation varies at each light intensity, see fig. 14); final (day 17), termination of experiment.

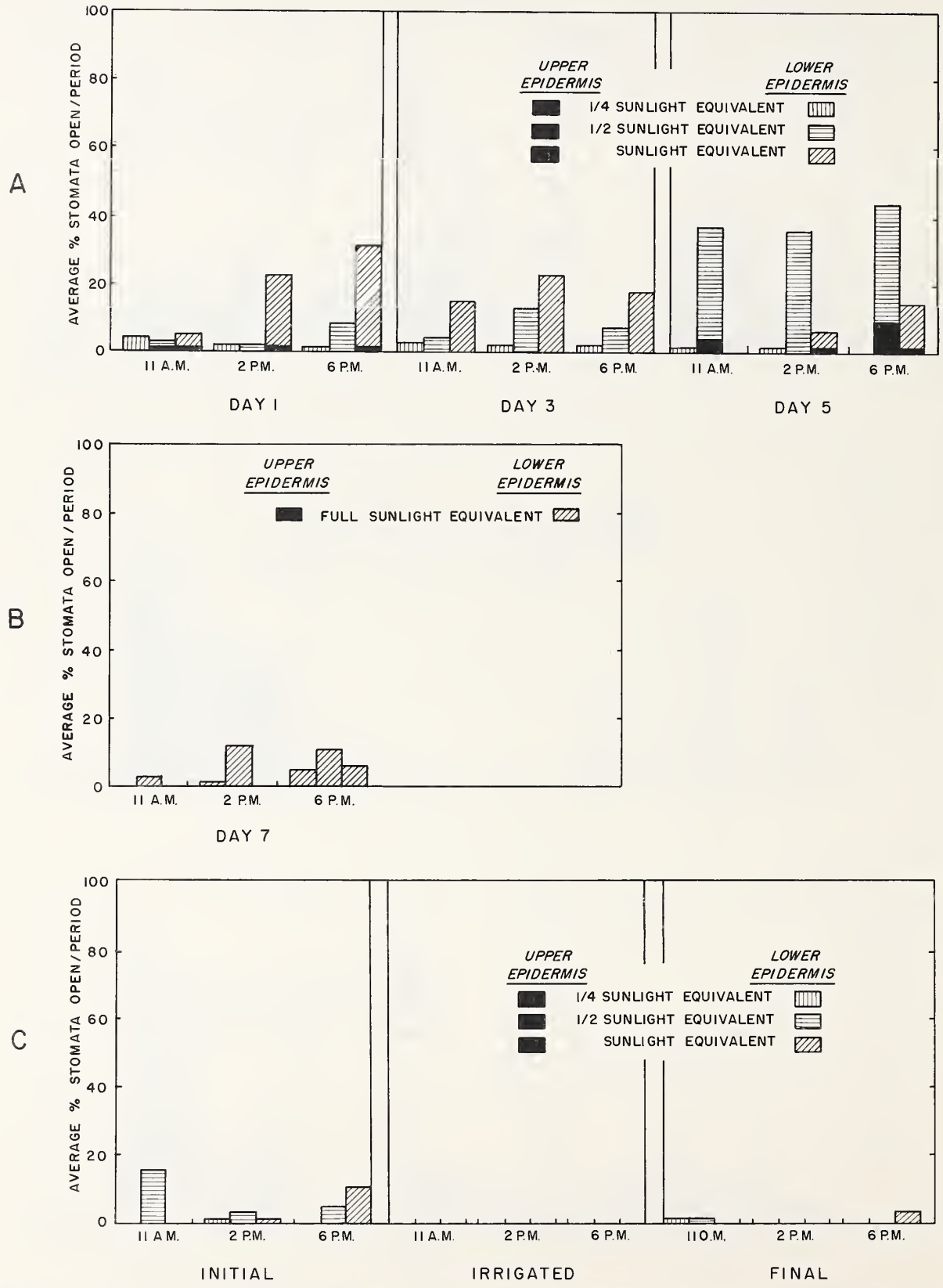

FIGURE 19.—Experiment 3. Percentage of stomata open on upper and lower epidermis of corn leaves at indicated light intensities. Individual bars are average of preceding 3 hours' activity. *A*, Preconditioning study; *B*, conditioning; *C*, soil moisture tension study: Initial (day 8); irrigated (day of reirrigation varies at each light intensity, see fig. 15); final (day 15), termination of experiment.

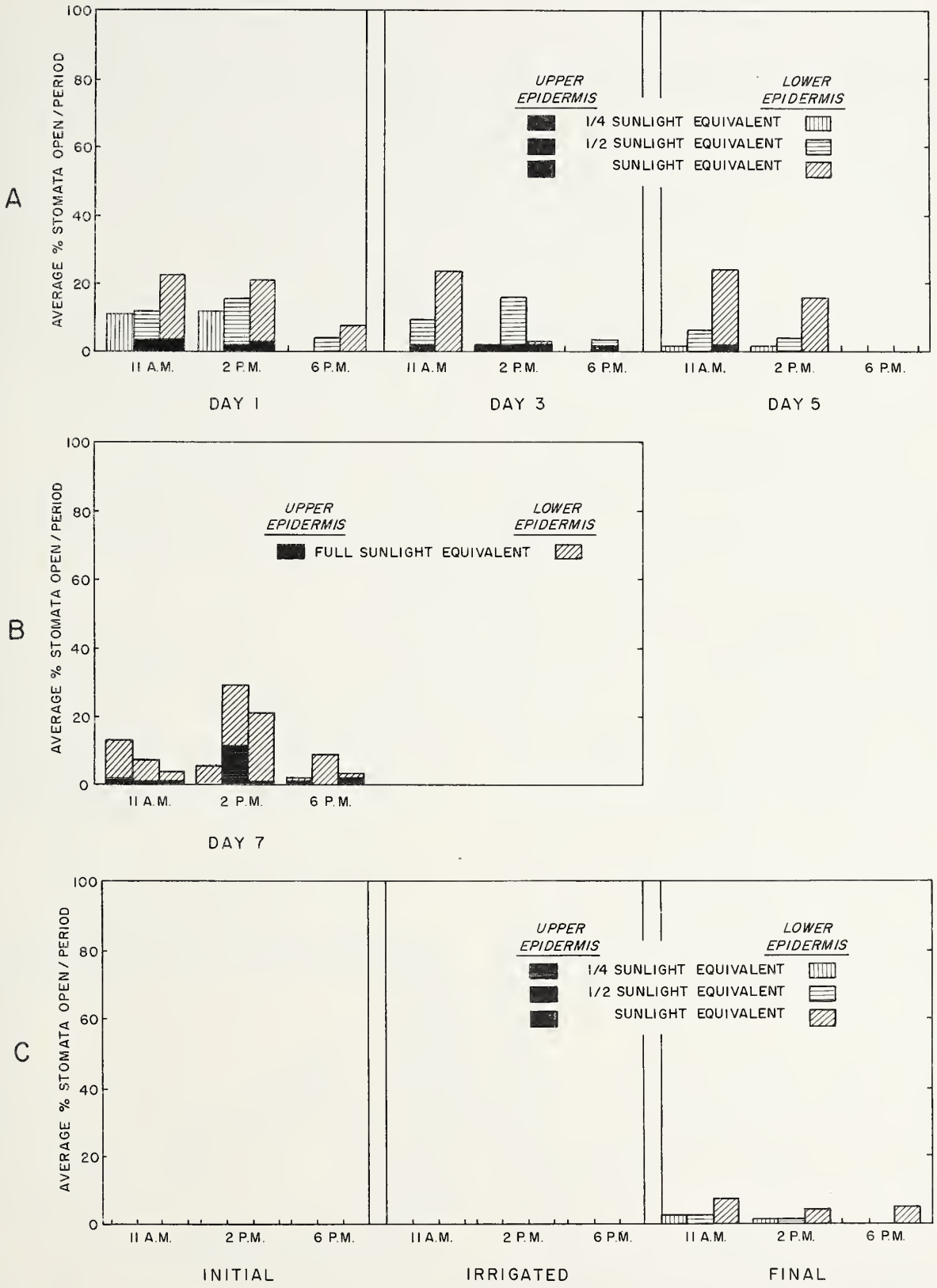

FIGURE 20.—Experiment 4. Percentage of stomata open on upper and lower epidermis of corn leaves at indicated light intensities. Individual bars are average of preceding 3 hours' activity. *A*, Preconditioning study; *B*, conditioning; *C*, soil moisture tension study: Initial (day 8); irrigated (day of reirrigation varies at each light intensity, see fig. 16); final (day 14), termination of experiment.

FIGURE 21.—Experiment 3. Apparent photosynthesis at indicated light intensities as affected by increasing soil moisture tension. Plants watered to near 0.05 atmosphere at 8 p.m. on day 7; depending on light intensity re-watered either at 9:15 a.m. on day 11 or 12 or at 10 a.m. on day 14.

FIGURE 22.—Experiment 4. Apparent photosynthesis at indicated light intensities as affected by increasing soil moisture tension. Plants watered to near 0.05 atmosphere at 8 p.m. on day 7 and rewatered at 10:30 a.m. on days 11, 12, and 13.

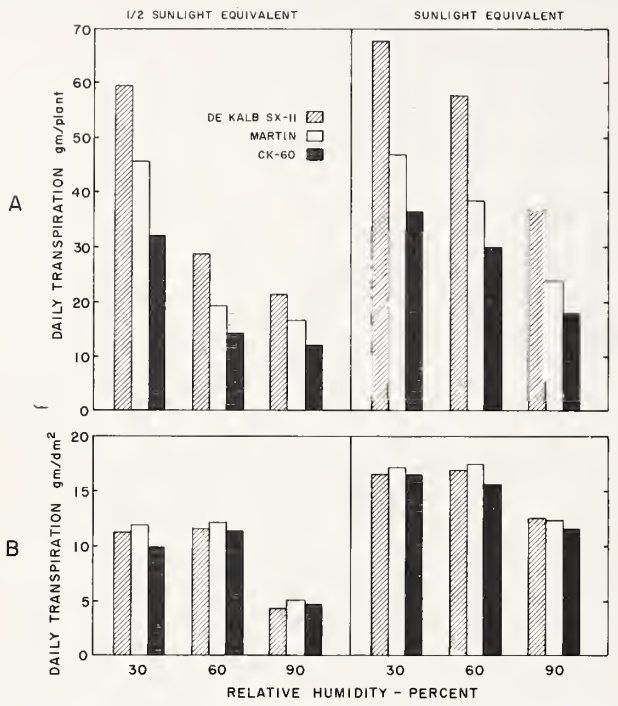

FIGURE 23.—Effect of light and humidity on daily transpiration by two grain sorghums and a grain sorghum-sudangrass cross during first week: *A*, Grams per plant; *B*, grams per square decimeter of leaf area.

two grain sorghums and the grain sorghum-sudangrass cross. Daily transpiration consisted of the water loss from 9 a.m. to 7 p.m. each day.

Statistical analysis revealed that for both the first and second weeks of the study there were highly significant differences between strains of sorghum when transpiration was expressed on a plant basis (figs. 23, *A*, and 24, *A*). However, when transpiration was expressed on a leaf-area basis (figs. 23, *B*, and 24, *B*), there was a significant difference between strains for only the first week of the study. This indicates that up to about 3 weeks of age something other than plant size caused differences in transpiration by the three strains. After about 3 weeks of age, transpirational differences between strains were due to differences in plant size. No explanation can be offered as to differences in transpiration by the young plants of the three strains.

The three strains showed a highly significant increase in transpiration at all three humidity levels when light intensity was increased from 0.65 to 1.35 cal. $cm.^{-2}$ $min.^{-1}$. This agrees with results obtained by Arthur and Stewart (*3*) with tobacco plants and indicates that light has a fairly direct effect on transpiration.

Observations during the second week of the study (fig. 25) showed that stomatal opening did not correspond closely enough to transpiration to substantiate Kramer's statement (*37*) that light affects transpiration primarily by its effect on stomatal activity. Very probably this response to light was due to a rise in leaf temperature, which increased the vapor pressure gradient from the leaf tissue to the atmosphere.

Figures 23, *B*, and 24, *B*, show that at both light intensities transpiration per unit leaf area decreased for the second week of the study. This was probably due to increased self-shading of the plants' lower leaves, which was evident on the older plants.

A highly significant *F* value for the interaction of light times humidity indicated that the plants did not show the same response to light at all humidity levels. During the first week (fig. 23, *B*), doubling the light intensity caused about 37-percent increase in transpiration per unit leaf area at both 30- and 60-percent relative humidity, but more than 150-percent increase at 90-percent relative humidity. In the second week (fig. 24, *B*), transpiration per unit leaf area increased by 88 and 125 percent at 30- and 90-percent relative humid-

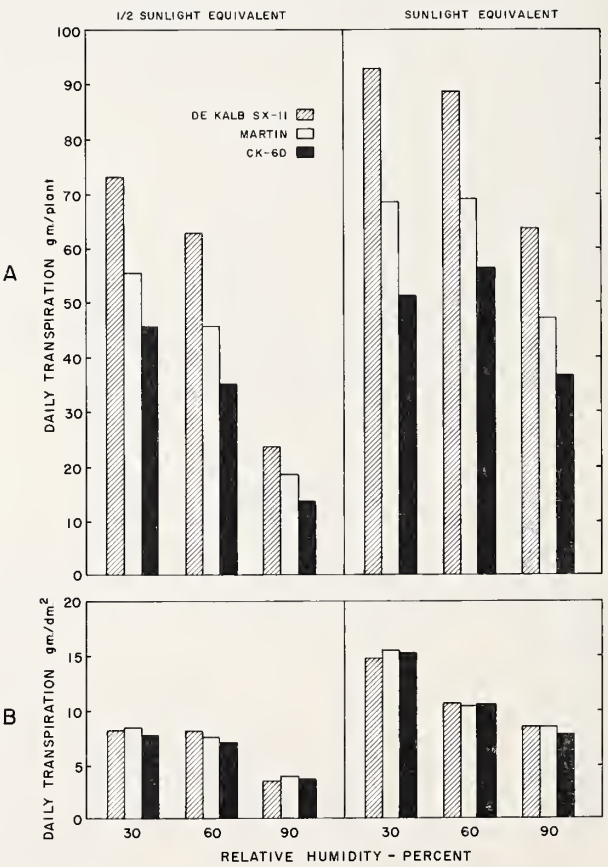

FIGURE 24.—Effect of light and humidity on daily transpiration by two grain sorghums and a grain sorghum-sudangrass cross during second week: *A*, Grams per plant; *B*, grams per square decimeter of leaf area.

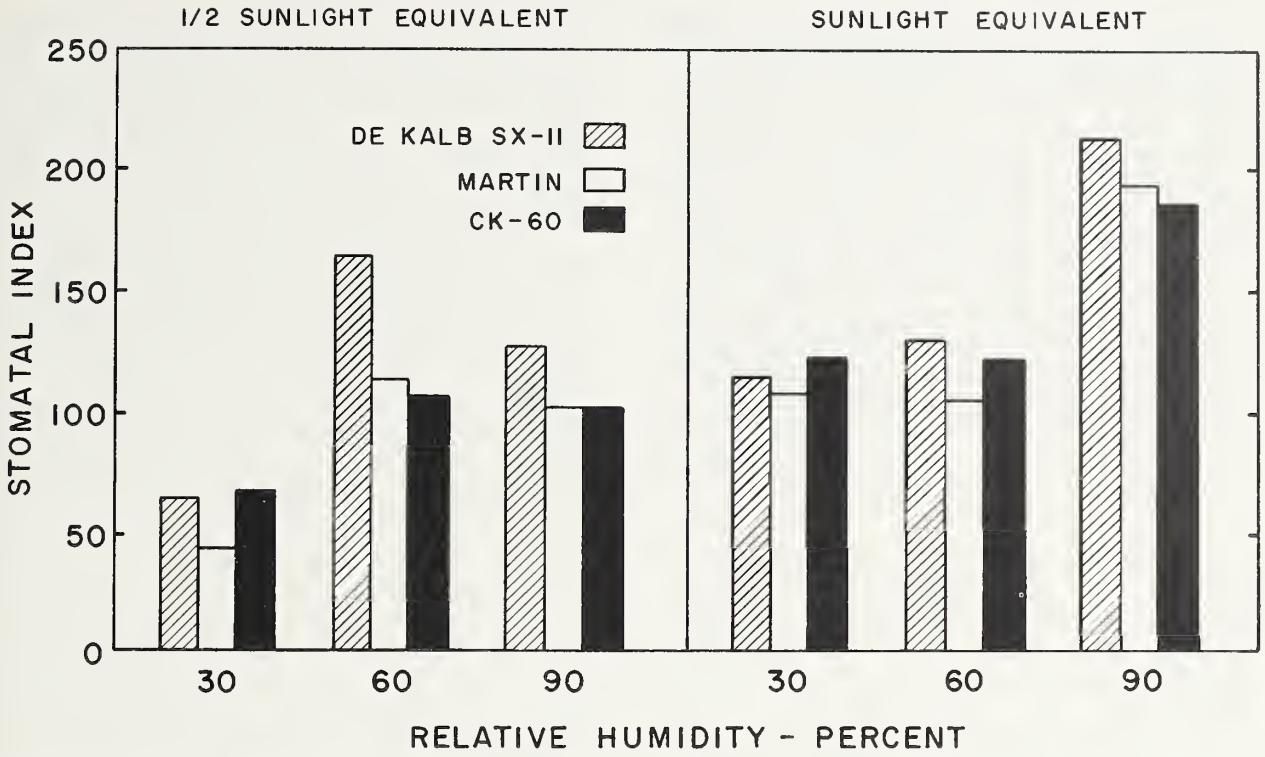

FIGURE 25.—Effect of light and humidity on stomatal index of two grain sorghums and a grain sorghum-sudangrass cross during second week.

ity, respectively, but increased by only about 39 percent at 60-percent relative humidity.

The only explanation that can be offered as to the unusual response to light at various humidities during the second week is based on changes in plant size that occurred during the week. Table 8 shows that the full light and 30-percent relative humidity happened to fall on Monday, whereas the one-half light and 30-percent relative humidity treatment fell on Saturday. During this time the leaf area of the plants (fig. 26) had increased by more than 50 percent.

Since transpiration per unit leaf area decreased as leaf area increased, the difference in transpiration between these two treatments was magnified. This explanation is substantiated by the fact that there was only a 22-percent increase in transpiration on a plant basis. The true response should be between that obtained on a plant basis and that obtained on a leaf-area basis, which would put the response to light at various humidity levels for the second week more in line with that of the first week.

Analysis of variance showed highly significant differences in transpiration due to relative humidity for both weeks of the study, both on a leaf-area and on a plant basis. At one-half light intensity for the first week there was considerable reduction in transpiration on a leaf-area basis when relative humidity was increased from 60 to 90 percent, but no difference between 30- and 60-percent relative humidity (fig. 23, *B*). However, figure 23, *A*, showing transpiration on a plant basis, indicates a large difference between 30- and 60-percent relative humidity. Again, this apparent discrepancy was due to changes in plant size that took place during

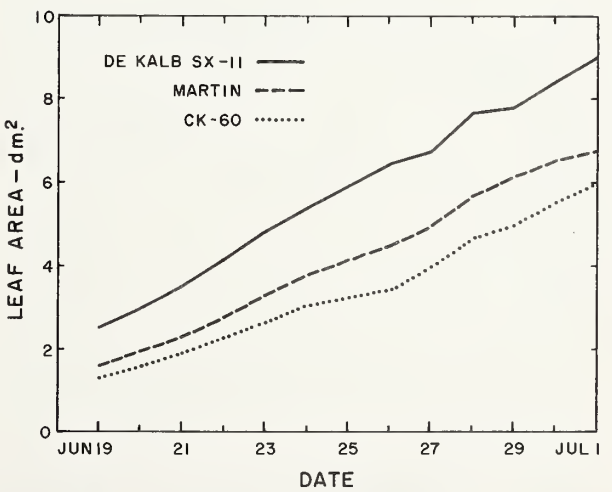

FIGURE 26.—Leaf area of two grain sorghums and a grain sorghum-sudangrass cross during first week.

the week. The 60-percent relative humidity treatment was on Monday and the 30-percent relative humidity treatment was on Saturday. The magnitude of the response is between that shown on a plant basis and that shown on a leaf-area basis.

At full light intensity during the first week there was less difference in transpiration between 30- and 60- than between 60- and 90-percent relative humidity, both on a leaf-area and a plant basis. In the second week there was definitely less response to humidity between 30- and 60- than between 60- and 90-percent relative humidity at both light levels. Indications are that under the conditions of this study transpiration by sorghum was affected more by changes from 60- to 90- than by changes from 30- to 60-percent relative humidity.

Stomatal behavior.—Daily stomatal index values for the two strains of sorghum and the sorghum-sudangrass cross as affected by two light levels and three relative humidities in the second week of the study are shown in figure 25. Stomatal index values were combined into daily values, since stomatal behavior of sorghum was fairly constant throughout each day.

Analysis of variance revealed highly significant differences in stomatal behavior between strains. Figure 25 shows that DeKalb SX–11, the grain sorghum-sudangrass cross, tended to have slightly greater stomatal activity than the other two strains.

Stomatal behavior, insofar as response to light and humidity were concerned, was very similar for

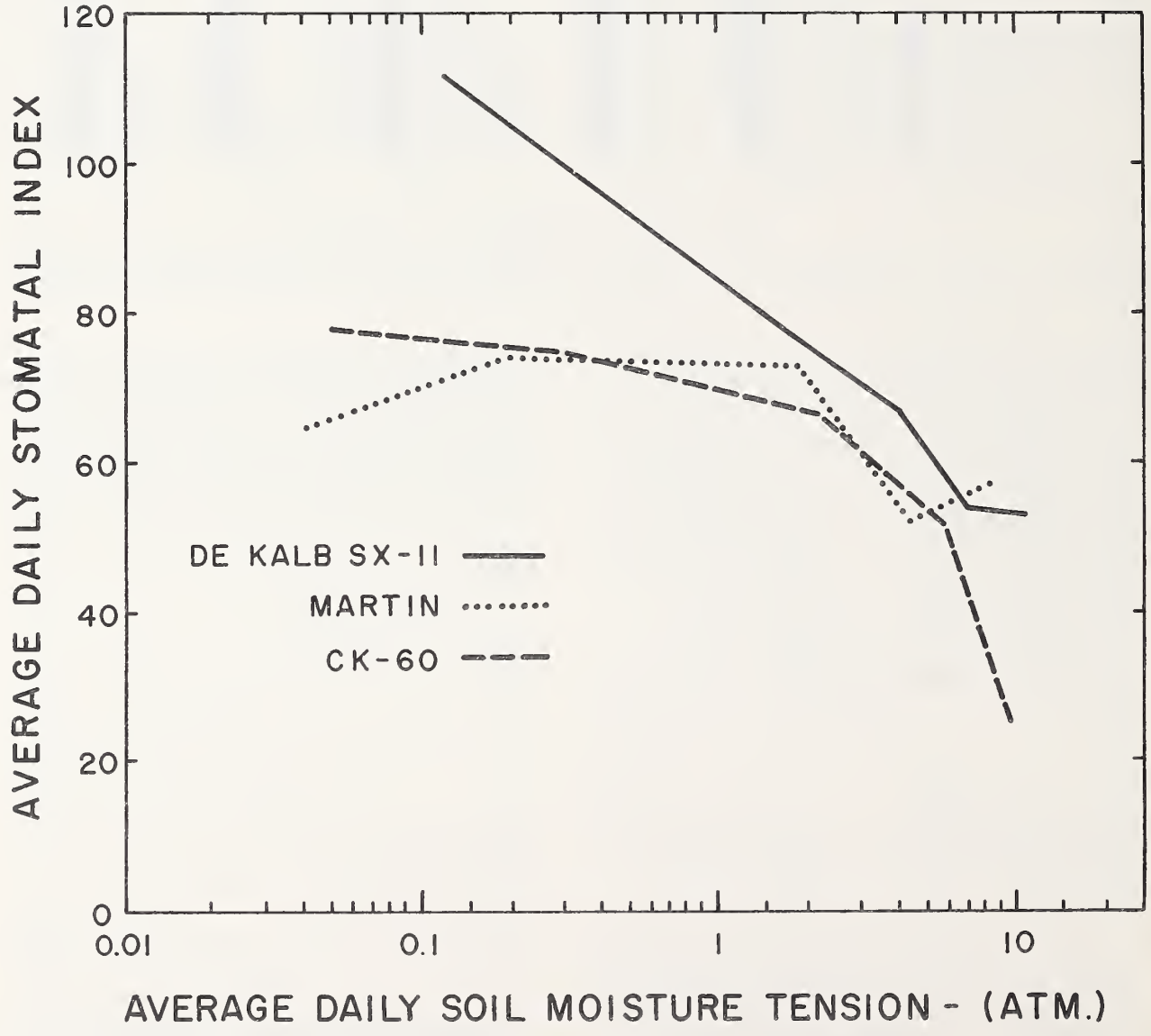

FIGURE 27.—Effect of soil moisture tension on stomatal index of two grain sorghums and a grain sorghum-sudangrass cross.

all three strains. The stomata of all three strains showed highly significant responses to light and humidity and to the interaction of these two variables. At both 30- and 90-percent relative humidity the stomatal index was approximately doubled when light was increased from one-half to full intensity. However, there was no response of stomatal behavior to light at 60-percent relative humidity.

Figure 25 shows that at one-half light intensity stomatal index increased when relative humidity was increased from 30 to 60 percent, with no further increase in stomatal index as relative humidity was increased to 90 percent. However, at full light intensity there was no response in stomatal index as relative humidity was increased from 30 to 60 percent, but there was an increase in stomatal index when the relative humidity was increased to 90 percent. No explanation can be offered for this interaction of the effect of light and humidity on stomatal behavior, unless it is partially explained by the effect of the previous day's treatment.

A highly significant simple correlation coefficient of 0.453 was obtained for the correlation of stomatal index with both the light and the humidity of the previous day. This is a fairly low correlation coefficient; however, the additive effect of the previous day's light and humidity may have had considerable effect on the plants' stomatal behavior. The reader can refer to table 8 to find the daily sequence of treatments, keeping in mind that these stomatal observations began on Monday of the second week.

Effect of soil moisture tension on stomatal behavior.—The effect of soil moisture tension on stomatal behavior is shown in figure 27. On these curves the average daily stomatal index is plotted opposite the average daily soil moisture tension for each of the three strains.

Stomatal behavior was very similar for Martin and CK–60, except at higher soil moisture tensions the stomatal index of CK–60 dropped well below that of Martin. At low tensions DeKalb SX–11 had a much higher stomatal index than Martin or CK–60, but when tensions increased to 2 or 3 atmospheres the stomatal index for all three strains was similar.

Soil moisture tension showed a highly significant correlation to stomatal index with r equal to -0.494. Stomatal index, in turn, had a highly significant correlation to transpiration with r equal to 0.590. These are low correlation coefficients; however, figure 27 shows that there was definitely a decrease in stomatal opening as soil moisture tension increased. Also, at the lower soil moisture tensions, DeKalb SX–11 had a slightly higher rate of transpiration per unit leaf area than the other two strains. (See *75, p. 17.*) In this same tension range, it had a higher stomatal index.

Effect of Fertility Level and Container Size on Transpiration, Growth, and Nutrient Uptake by Grain Sorghum

The fertility requirement for a soil used in the environment control room is greater than it is for the same soil in the field, because the rate of plant growth is high and the roots are in contact with a relatively small volume of soil. When the rate of fertilization is increased to supply all the nutrients required for plant growth, the probability of creating toxic levels of certain ions is increased. The objective of this experiment was to study the effect of fertility level and container size on plant growth, uptake of nutrients, and transpiration of grain sorghum (*Sorghum vulgare* Pers.) in a controlled environment.

Procedure

The environmental conditions for this study were 14 hours' light, temperature 25° C., relative humidity 60 percent; and 10 hours' dark, temperature 20°, relative humidity 90 percent. A light intensity of 0.85 cal. $cm.^{-2}$ $min.^{-1}$ was used throughout the study. The experiment was a 5 by 4 by 2 factorial, using five rates of potassium (0, 62, 125, 250, and 500 p.p.m.) as potassium chloride, four rates of phosphorus (0, 25, 50, and 100 p.p.m.) as 20-percent superphosphate, and two container sizes (3,600 and 1,800 gm. of soil).

When the plants were 2 weeks old, the collection of data was initiated. Transpiration rate per unit of leaf area was determined by weighing the container at 2-hour intervals and measuring the leaf area each day. The dry-matter yield and the phosphorus, potassium, and chloride content of the dry matter were determined.

Results and Discussion

The dry matter produced in the large containers was twice that produced in the small containers. Plant growth increased as the rate of phosphorus added was increased and decreased as the rate of potassium chloride added was increased. The effect of phosphorus and potassium chloride on yield was the same regardless of container size, as shown in table 9.

Average transpiration rate per day per unit of leaf area is given in table 10. Transpiration rate per leaf area in the large and small containers increased as the rate of phosphorus added was increased and decreased as the rate of potassium chloride added was increased. Transpiration rate per leaf area was greater for the plants in the large containers than for the plants in the small containers. The effect of added phosphorus and container size on transpiration rate was highly significant. The decrease in transpiration rate due to the potassium chloride

TABLE 9.—*Effect of phosphorus and potassium chloride added and container size on dry-matter yield*

Potassium chloride added (p.p.m.)	Dry-matter yield when indicated amount of phosphorus (p.p.m.) was added to—							
	Large containers				Small containers			
	0	25	50	100	0	25	50	100
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
0	3.75	5.25	8.10	9.90	2.35	2.55	3.90	5.65
118	3.55	4.05	7.05	9.50	2.05	2.00	4.20	5.25
236	1.30	3.75	6.30	10.30	2.00	2.95	3.45	5.10
472	1.60	2.30	5.65	8.75	1.20	1.90	3.40	4.70
944	.90	2.50	4.55	5.25	.85	1.60	2.25	4.45

TABLE 10.—*Effect of phosphorus and potassium chloride added and container size on transpiration rate*

Fertilizer added (p.p.m.)	Water transpired per leaf area in—	
	Large container	Small container
	PHOSPHORUS	
	Gm./dm.²/day	*Gm./dm.²/day*
0	16.5	15.1
25	18.1	16.3
50	19.3	18.3
100	19.6	18.6
	POTASSIUM CHLORIDE	
0	19.1	17.9
62	18.1	17.8
125	18.1	17.2
250	18.5	16.7
500	18.0	16.6

added was not statistically significant at the 5-percent level.

The percentage of phosphorus in the plant was not appreciably affected by the potassium chloride rates, but decreased as the phosphorus rates were increased, as shown in table 11. The percentage of potassium in the plant increased as the potassium chloride rates were increased and decreased as the phosphorus rates were increased. The percentage of chloride in the plant increased as the potassium chloride was increased, but it was not affected by the phosphorus rates.

Roots were removed to study the effect of treatment on root growth. The higher rates of potassium chloride resulted in the development of a poor root system. This was attributed to the toxicity of the chloride ion. As the phosphorus rates increased, plants developed a more prolific root system. The transpiration rate of a plant in a given environment can be increased or decreased by a soil amendment that will increase or decrease the root growth or volume of absorbing surface.

The fertilization rate presently used for major growth room studies (see p. 5) appears quite adequate.

GUARD CELL ACTION

Osmotic Pressure Determinations

Previously reported (*51*) values of guard cell osmotic pressure (OP) with *Erigeron philadelphicus* L. did not unequivocally substantiate increases of OP with stomatal opening or decreases of OP with stomatal closure. A major difficulty with *Erigeron* was the high variability in stomatal opening and OP of guard cells on different leaves and even on the same leaf at a given time. Such variability as reflected in stomatal opening is depicted in the series of pictures in figure 28, *A*. Each picture is typical of the average stomatal condition in that section of the leaf (fig. 28, *B*), and the variability as shown ranges from full open to complete closure. Similar findings by us and by Loftfield (*40*) with a large number of species have prompted the use of percentage of open stomata in our reporting instead of average pore diameters or some other seemingly definite measurement.

The OP of guard cells has been considered of primary importance in the mechanism of guard cell action; therefore, the substantiation of a midday drop in OP (*51*) needed clarification. An interesting approach considered was that of determining OP throughout a diurnal cycle. Such determinations might result in further leads. Since the environmental factors in the field are both unpredictable and uncontrollable, OP de-

TABLE 11.—*Effect of phosphorus and potassium chloride added and container size on amount of phosphorus, potassium, and chloride in grain sorghum*

PHOSPHORUS

Potassium chloride added (p.p.m.)	Amount of nutrient in plant when indicated amount of phosphorus (p.p.m.) was added to—															
	Large container								Small container							
	0		25		50		100		0		25		50		100	
	Percent	*Mgm.*	*Percent*	*Mgm.*	*Percent*	*Mgm.*	*Percent*	*Mgm.*	*Percent*	*Mgm.*	*Percent*	*Mgm.*	*Percent*	*Mgm.*	*Percent*	*Mgm.*
0	0. 33	1. 23	0. 36	1. 89	0. 22	1. 78	0. 19	1. 83	0. 29	0. 67	0. 29	0. 73	0. 18	0. 70	0. 18	1. 02
118	. 31	1. 08	. 32	1. 30	. 23	1. 62	. 20	1. 89	. 27	. 54	. 32	. 64	. 20	. 84	. 19	1. 00
236	. 26	. 27	. 40	1. 48	. 23	1. 42	. 22	2. 26	. 27	. 54	. 27	. 80	. 19	. 65	. 19	. 97
472	. 22	. 35	. 30	. 69	. 24	1. 34	. 22	1. 88	. 29	. 35	. 30	. 57	. 20	. 68	. 19	. 88
944	--------	------	. 22	. 54	. 24	1. 09	. 24	1. 25	. 25	. 21	. 27	. 43	. 23	. 51	. 17	. 75
	POTASSIUM															
0	4. 1	15. 4	3. 0	15. 8	1. 8	14. 6	0. 2	1. 5	3. 6	9. 0	4. 0	10. 5	2. 0	7. 6	1. 4	7. 9
118	4. 7	16. 7	3. 7	15. 3	3. 2	22. 5	2. 6	24. 5	4. 1	8. 4	2. 1	4. 2	2. 5	10. 7	1. 9	10. 0
236	3. 7	3. 5	4. 7	17. 6	2. 0	12. 3	3. 2	33. 0	4. 2	8. 5	4. 4	13. 1	3. 5	11. 9	3. 4	17. 6
472	2. 9	3. 7	4. 4	10. 5	3. 9	21. 5	3. 9	34. 1	--------	------	4. 2	8. 0	3. 8	13. 4	3. 5	16. 8
944	1. 8	1. 6	4. 6	11. 5	4. 8	21. 8	4. 4	22. 9	--------	------	4. 7	7. 6	4. 1	9. 2	3. 8	18. 2
	CHLORIDE															
0	0. 52	1. 94	0. 67	3. 54	0. 49	3. 97	0. 35	3. 50	0. 48	1. 20	0. 62	1. 56	0. 44	1. 70	0. 36	2. 02
118	. 96	3. 51	1. 05	4. 31	1. 07	7. 40	1. 07	10. 12	. 76	1. 56	. 86	1. 72	. 94	3. 95	. 91	4. 77
236	1. 35	1. 34	1. 13	4. 25	1. 16	7. 36	1. 14	11. 18	1. 11	2. 24	1. 07	3. 15	1. 13	3. 43	1. 13	5. 70
472	1. 11	1. 78	1. 35	2. 85	1. 16	6. 50	1. 23	10. 8	1. 07	1. 61	1. 02	1. 94	1. 15	3. 96	1. 25	5. 61
944	--------	------	1. 33	3. 32	1. 53	6. 98	1. 43	7. 43	1. 31	1. 12	1. 52	2. 42	1. 48	3. 34	1. 10	4. 95

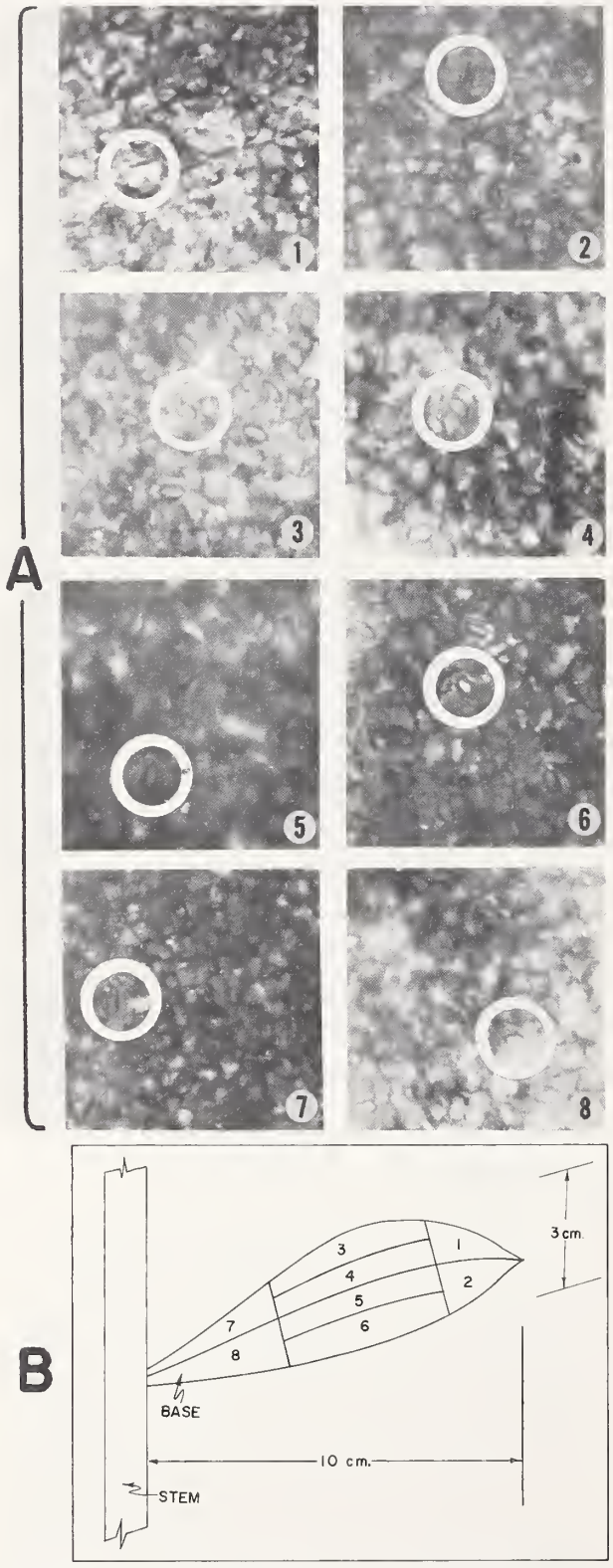

FIGURE 28.—*A*, Stomatal condition on lower epidermis at 2 p.m. in eight sections of mature *Erigeron philadelphicus* leaf; *B*, code for sections illustrated in *A*.

terminations under controlled conditions were considered most worthwhile.

Two good subjects were *Rheum rhaponticum* L. (rhubarb) and *Vinca major* L. Both species appear to grow equally as well in shade or sun, they have large guard cells so that microscopic observation is easy, and, most important to OP determinations, the lower epidermis strips easily. Also, stomatal checks in the greenhouse indicated that the guard cells of both species responded somewhat uniformly to changing environmental conditions.

The optimal environmental conditions, such as light and humidity, for guard cell operation of the species were unknown and needed determination before undertaking osmotic pressure checks. Therefore, mature plants were grown under several environmental regimes and stomatal operation was observed. Full and one-half sunlight equivalent and low, medium, and high humidity were the variables. Six representatives of the two species actively growing in the greenhouse were transferred to the growth room. Light quantity and humidity were changed as indicated in figure 29. Day temperature remained constant at 25° C. and photoperiod was 12 hours. Night temperature was 20° and humidity 90 percent. Plants were insured optimum soil moisture availability by frequent watering.

A mature leaf was chosen for stomatal observation from each of two representatives of each species. Stomatal counts were made in several interveinal sections marked with minute drops of India ink. This allowed the same stomata to be observed repeatedly. Fifty stomata were recorded at each location as to the closed or open condition. The counts were made from 8:30 to 9 a.m., 12:30 to 1 p.m., and 4:30 to 5 p.m.

The operation of *Vinca* stomata was inconsistent under the growth room conditions (fig. 29), whereas rhubarb stomata behaved more consistently. The one-half light intensity at medium and high humidity resulted in the best overall stomatal and plant response. Full light caused wilting of rhubarb leaves even under high humidity, possibly bringing about some passive opening (*51*). Low humidity with one-half sunlight also caused measurable wilting.

A low stomatal activity was recorded on July 3 under environmental conditions comparable to the previous and following days. Although this record was puzzling at the time of the experiment, research since then explains such discrepancies.

For this experiment the leaves of rhubarb had a decided advantage because they were large. A single leaf could serve for OP checks for several days and, therefore, should minimize inherent differences between leaves mentioned earlier.

Based on the environmental information obtained, osmotic pressure changes and stomatal reg-

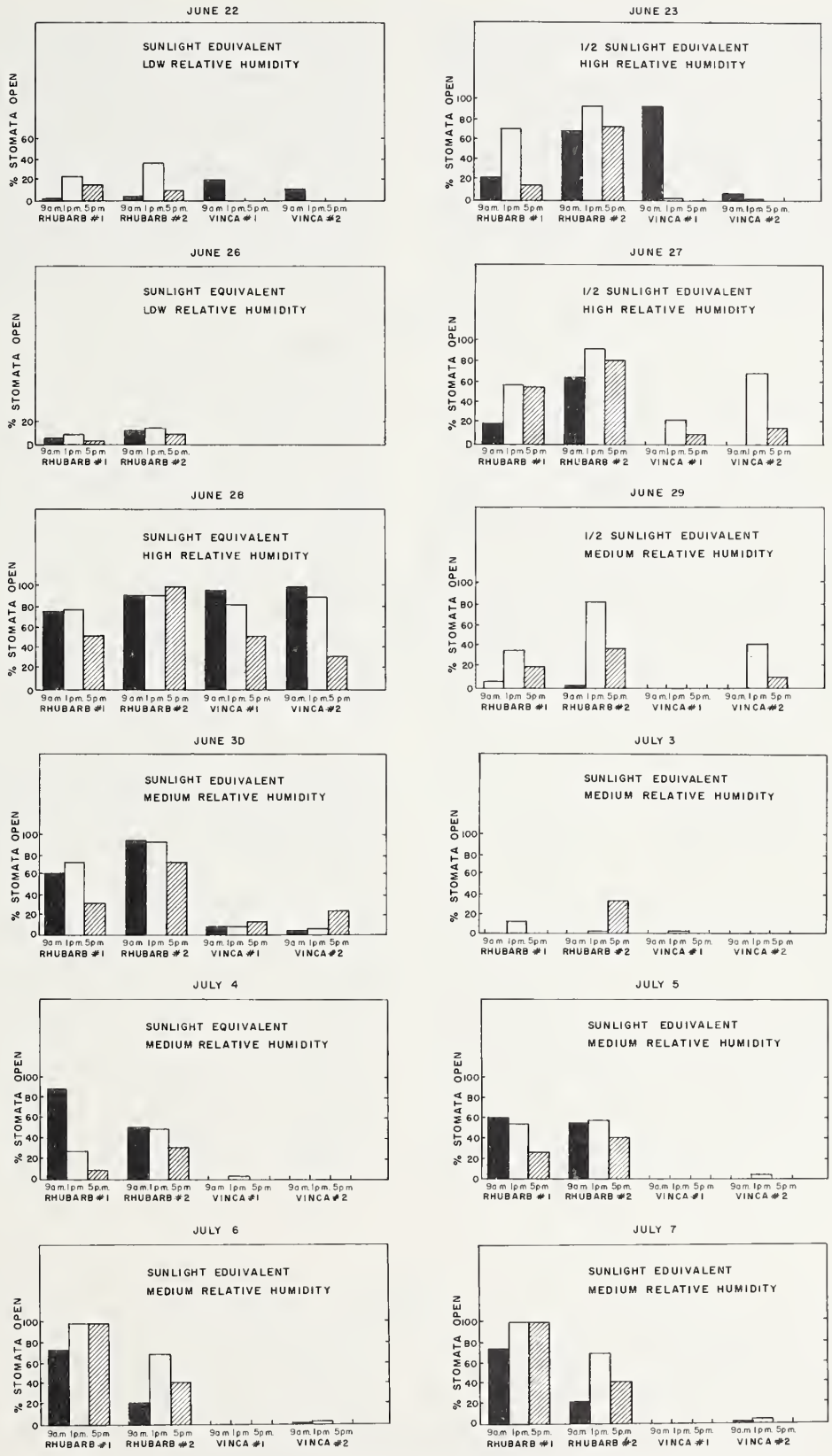

FIGURE 29.—Percentage of stomata open at indicated light intensity and humidity on lower epidermis of *Rheum rhaponticum* (rhubarb) and *Vinca major*.

ulation by guard cells of rhubarb were followed through several diurnal cycles at one-half sunlight intensity. The optimum humidities in the previous study were used, consisting of high (90 percent) and medium (60 percent) for experimental periods of 36 hours each. Rhubarb plants were brought from the greenhouse into the growth room. During the first day the plants were conditioned to one-half sunlight, 25° C., and 90-percent relative humidity during a 12-hour photoperiod.

The following determinations were started at 8 a.m. on day 2:

(1) Percentage of open stomata in each of six equal sections of each of two leaves at 4-hour intervals, continuing uninterrupted for 84 hours.

(2) Continuous osmotic pressure determinations of guard cells from one of the sections of each leaf at midnight and noon.

The incipient plasmolysis method (*17*) was used to determine OP. Sucrose was the plasmolyzing agent. To derive the curves in figure 30, run 1,

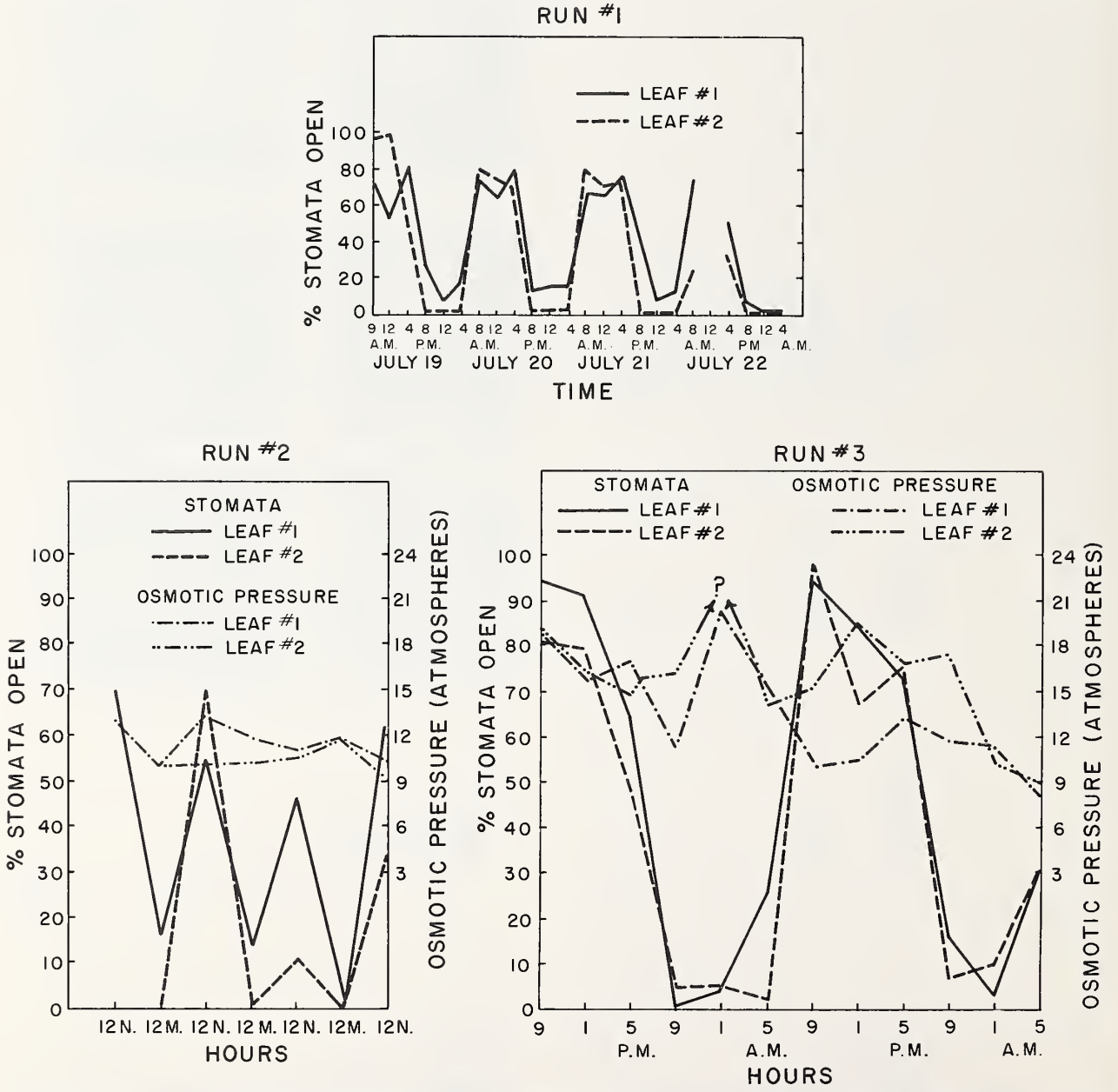

FIGURE 30.—Percentage of stomata open at indicated times on lower epidermis of *Rheum rhaponticum* leaves (runs 1–3) and their osmotic pressure (runs 2–3).

considerably more than 1,000 cells were observed at each period. Throughout the microscopic work the variability in different sections of the same epidermal peeling was striking and at the same time was not convincing to the observer that results gathered by such a method were irrefutable.

The general tendency of stomatal action was the opened condition during the light hours and closure during the dark hours; however, complete closure did not occur at night on leaf 1 (fig. 30, run 1) and is shown quite strikingly for the individual section on which the OP measurements were taken (fig. 30, run 2).

A most interesting finding was the lack of diurnal changes in the measured osmotic pressure. Guard cells of leaf 2 maintained a rather consistent OP value at the same time that extremes of stomatal condition were recorded. With high humidity, guard cells of leaf 2 showed a rather consistent high OP value at noon of the first experimental day and a low value at midnight; the trend continued for 2 days, but disappeared after the humidity was lowered. A midday drop in OP as reported in *Erigeron* (*51*) was not found in this study. In summary then, opening and closing movements of stomata continued without correlating with OP changes. These findings warranted further experimentation.

In searching the literature for possible leads to explain our results of no correlation between OP and stomatal operation, the report (*65*) was noted that sucrose was not an ideal plasmolyzing agent. It was not understood how its use could produce our results, but for the sake of accuracy several experiments of guard cell plasmolysis were run in which the four commonly used plasmolyzing agents were compared; namely, sucrose, mannitol, sodium chloride (NaCl), and potassium nitrate (KNO_3). Sucrose, mannitol, and NaCl gave the most consistent results. NaCl induced a little sharper plasmolysis at times; KNO_3 frequently gave indistinct plasmolysis.

A third run still using sucrose was set up on two more greenhouse-grown rhubarb plants. Not only was stomatal operation monitored every 4 hours in this study but also osmotic pressure determinations were simultaneously made. Each leaf was divided into 10 sections for the observations and determinations. The temperature and photoperiod were the same as used previously; however, only 90-percent relative humidity was used.

A similar stomatal operational trend of open in the light and closed in the dark with a little overlapping occurred in this study (fig. 30, run 3). Any osmotic pressure changes were still not delineated. Leaf 1 showed a decrease in OP at the end of the light period of the first day, whereas leaf 2 indicated a drop in OP toward the end of the day and a rise after dark. The measurement taken at 1 a.m. was most disconcerting—a very high OP value was obtained. Another sampling as well as a change in plasmolyticum substantiated the value.

A check with iodine potassium iodide showed no distinct grains of starch in the guard cells, but rather a diffuse red amorphous staining reaction throughout the cytoplasm, as already found under different circumstances (*51*, *fig. 6*). The value for leaf 2 never was determined for 1 a.m., as it exceeded the highest value of plasmolyticum used. No dramatic drop in OP occurred during the night hours. A low OP value was found the next morning on both leaves, rising throughout the day.

The plasmolyticum was changed from sucrose to NaCl at 5 p.m. on the last day of this study. This change was made as the possibility existed that sucrose was penetrating the guard cells at night and thus effectively increased the OP of the guard cell. This postulation may be partially true, as no peak in OP during the night hours was found after the plasmolyticum change. The OP remained high after dark for awhile, but it fell continually as the night progressed (fig. 30, run 3).

Guard Cell Starch Retention and Accumulation in the Dark

Studies by Pallas (*50*) have shown that the guard cells of 48 living plant species retain starch for prolonged periods of darkness. When strips of epidermis were floated on sugar solution in the dark, a number of externally supplied sugars acted as precursors to guard cell starch production. Studies with the test sugars showed that mesophyll and bundle cells were involved in uptake, transformation, and translocation.

EFFECTS OF CERTAIN CHEMICALS ON TRANSPIRATION

Since the initiation of this cooperative project in 1959, an increased interest has developed in the potential of reducing transpiration by means of transpiration suppressants. This is exemplified by the recent increase in publications and the National Science Foundation-sponsored seminar on the subject (July 1963, Connecticut Agricultural Experiment Station, New Haven, Conn.). We have reported (*51*) no significant reduction in transpiration by 2,4-dichlorophenoxyacetic acid

[2,4-D], N^6 benzyladenine, hexadecanol, or carbowaxes that was not correlated with decreased plant growth.

Gale (*27*) in Israel has reported favorable results with vinyl acetate-acrylate esters, and other workers indicate that phenylmercuric acetate (*80*, *81*) and hydroxysulfonates (*67*, *78*) show promise. Reference to some of the early work on antitranspirants has already been made (*51*) with emphasis on the growth regulator aspect; however, several reports of additional research not mentioned specifically here should be noted by those interested (*1*, *8*, *41*, *44*, *45*, *62*, *70*).

In 1962 several compounds providing a physical barrier to transpiration or a potential control of stomatal operation were tested and the results are summarized here. This work does not support the highly optimistic view of controlling all transpiration, but it does point out a definite potential and the need for much more research on the subject.

Latex and Plastic Compounds

Several compounds are presently marketed by commercial companies (table 12). These compounds, which are latexes, waxes, and plastics, are generally applied in foliar sprays or dips. Some are widely used by horticulturists for reducing transpiration of transplants, bulbs, and Christmas trees. High costs of these compounds prevent extensive use in the fields of forestry and agronomy. There is also a paucity of published information on the efficiency of the compounds as antitranspirants. For this reason it was deemed necessary to evaluate them as potential transpiration suppressants.

Specific information pertinent to each compound tested was supplied by the manufacturer and is included in the appendix. These materials were tested at the manufacturer's recommended rate of dilution on beans, *Phaseolus vulgaris* L. variety red kidney, and on corn, *Zea mays* variety Dixie 82. These plants grow and transpire rapidly; therefore, they are excellent for evaluation studies. Transpiration before and after spraying was obtained by weight differences.

The general growth and test conditions of the experimental populations were the same as previously reported (*51*, *pp. 25–26*), except where specific variations are stated. To test the compounds, two separate populations each of corn and bean plants were grown and treated in different growth chambers. The bean plants were first treated when 30 days old and re-treated when 40 days old. Corn received only one spray application when 23 days old.

Figure 31, *A* and *B*, indicates that all latex and plastic compounds reduced bean transpiration on the day of treatment, and with most of the compounds the effect was prolonged at least 1 day thereafter. This early reduction is probably related to transpiration suppression with a minimum growth effect. The decreased transpiration suppression with increased time after treatment is probably related to growth disrupting surface films.

Best overall transpiration suppression was obtained with the Wilt Pruf and Sun Wax W formulations. However, under the experimental conditions, Rutex, Latex 5229 and 5230, Wilt Pruf, and Sun Wax W all reduced transpiration for several days after treatment.

The corn experiment (fig. 31, *C* and *D*) was prematurely terminated by a failure of power to the cooling compressors. Energy from the lights raised growth chamber temperatures to 130° F. The elevated temperature killed treated leaf tissue, but it did not injure the controls. This finding prompted the studies of antitranspirant effects on leaf temperature reported in a subsequent section.

TABLE 12.—*Antitranspirants tested*

Primary ingredient	Trade and experimental name	Recommended dilution	Company
Polyvinyl chloride	Wilt Pruf	4 : 1 v/v	Nursery Specialty Products, New York, N.Y.
Acrylic type polymer	Rutex W–3	1 : 3 or 4 v/v	UBS Chemical Co., Cambridge, Mass.
Latex (vinyl resin)	Vanex	1 : 4 v/v	Interchemical Corp., Englewood, N.J.
Latex (styrene butadiene copolymer).	Latex 5229	1 : 5 to 10 v/v	Miller Chemical & Fertilizer Corp., Baltimore, Md.
Latex (vinylidine chloride homopolymer).	Latex 5230	1 : 5 to 10 v/v	Do.
Paraffin wax	Sun Wax W	1 to 4 percent w/w	Sun Oil Co., Philadelphia, Pa.
Paraffin wax (60-percent emulsion)	Sun Wax 60	1 to 4 percent w/w	Do.

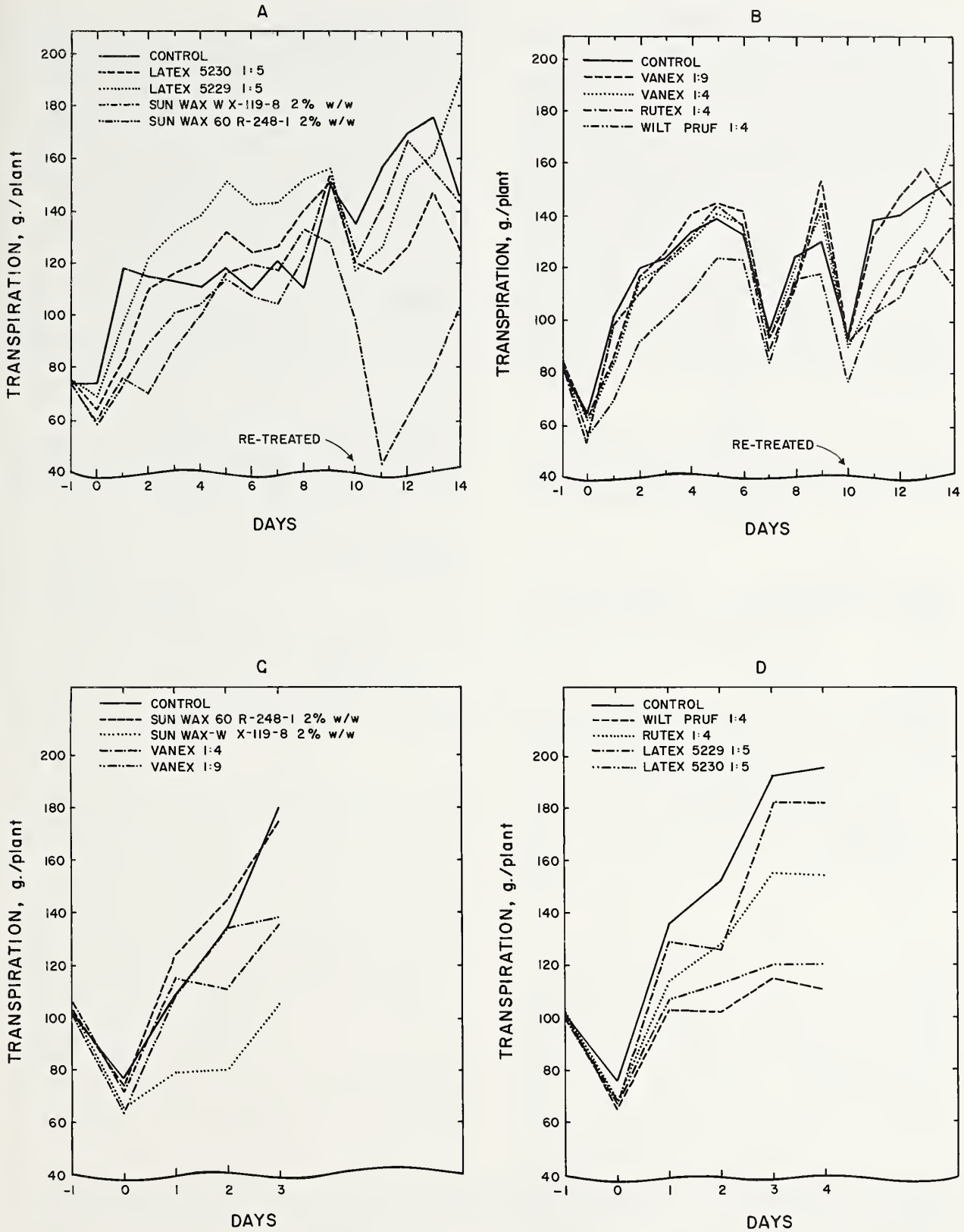

FIGURE 31.—Transpiration of red kidney bean plants (*A* and *B*) and Dixie 82 corn plants (*C* and *D*) sprayed at indicated concentrations with latex and plastic compounds (water to formulation v/v except for Sun Waxes).

Polyethylene

Polyethylene could be an ideal compound for transpiration control because of its imperviousness to water and permeability to oxygen and carbon dioxide. However, no marketed formulation was found; therefore, two formulations of polyethylene were prepared as polyethylene emulsions in our laboratory. In reply to our inquiry, Atlas Chemical Company recommended that we test formulas 3 and 6 as outlined in their Production Information Bulletin LG–72, 1960. The experimental polyethylene emulsions were lethal to 21-day-old bean plants when sprayed at full strength or 1:4 v/v dilution. Even though further testing was abandoned, these results are not conclusive proof that safe formulations cannot be made, because the effects of the surfactants were not evaluated. The surfactants may have been the toxic ingredients.

Mercury and Fluoride Compounds

In the search for compounds that would exert their effect on the enzymatic operation of guard cells, two appear paramount—mercuric chloride ($HgCl_2$) and sodium fluoride (NaF). $HgCl_2$ (10^{-3} M) is an inhibitor of amylase (*47*) and NaF (10^{-2} M) is an inhibitor of phosphatase (*49*). Both compounds are reported to accelerate the formulation of starch (*35*, *48*). It was postulated that if starch decomposition could be curtailed, reduction of stomatal operation might be possible. This hypothesis was based on the fact that starch performs an integral part in guard cell operation, although its exact function is not known (*51*). It may serve as the source for energy and also as an osmotically active material necessary to guard cell operation.

Two kidney bean populations were grown and sprayed with 10^{-3} and 10^{-2} M NaF, 10^{-3} and 10^{-2} M $HgCl_2$, and all combinations of the two. NaF alone had no measurable effect on transpiration (fig. 32, *A*). $HgCl_2$ by itself and in combination with NaF did reduce transpiration (fig. 32, *A* and *B*). However, it was also very phytotoxic at 10^{-2} M and slightly phytotoxic at 10^{-3} M.

Because transpiration was considerably reduced by $HgCl_2$ for an extended period, the percentage of open stomata on two mature leaves of each bean plant was checked microscopically 3 days after

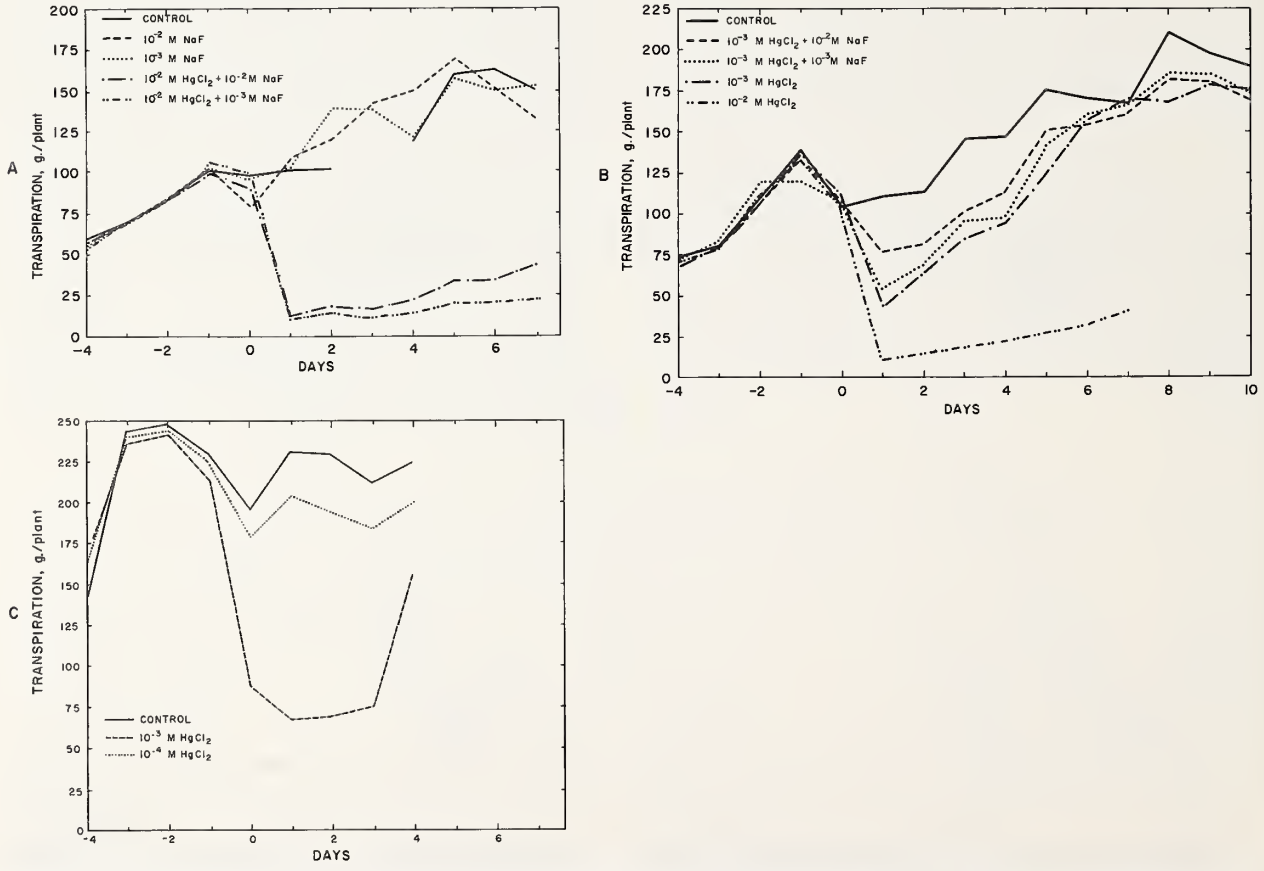

FIGURE 32.—Transpiration of red kidney bean plants sprayed at indicated concentrations with sodium fluoride (NaF) and mercuric chloride ($HgCl_2$): *A*, With NaF or $HgCl_2$ and NaF; *B*, with $HgCl_2$ or $HgCl_2$ and NaF; *C*, with $HgCl_2$.

treatment at 11 a.m. and 3 p.m. and on the fourth day at 9 a.m. No significant difference in the percentage of open stomata on the control versus the 10^{-3} M-treated plants could be found.

Although the foliar application of 10^{-3} M $HgCl_2$ resulted in a significant reduction in both transpiration and phytotoxicity, the effects of lower concentrations were unknown. Therefore, a 10^{-4} M application was considered of worth. The previous control plants were the subjects of the test and were sprayed with either distilled water or 10^{-3} or 10^{-4} M $HgCl_2$. Only a slight reduction of transpiration was observed with 10^{-4} M, but a decided reduction was again obtained with 10^{-3} M $HgCl_2$, as indicated in figure 32, *C*. Phytotoxicity was noted at 10^{-3} M. The symptom of toxicity at this concentration was small necrotic areas, less than a millimeter in diameter, mostly on lower leaves.

Another population of red kidney bean plants was sprayed at 21 days of age to establish more precisely the effective $HgCl_2$ threshold concentration and the transpiration of such treated plants to water stress. In this application 0.66 by 10^{-3}, 0.33 by 10^{-3}, 10^{-3}, and 10^{-4} M $HgCl_2$ solutions were used. Figure 33 indicates that the reduction in transpiration the day after application was again dependent on concentration; it was most effective at 10^{-3} M as previously found.

Soil moisture tensions were imposed in this experiment and were obtained by watering to near 0.05 atmosphere on the day of treatment, with no water added until 4:30 p.m. on day 2. Transpiration trends after rewatering were peculiar, in that the previously clear demarcation of transpiration between treatment rates was not evident on days 3 and 4.

Since the trends just mentioned immediately after treatment were in agreement with the previous study and since in that study stomatal opening was not found to be affected by the $HgCl_2$, the possibility existed that $HgCl_2$ exerted its effect in the root area by curtailing water absorption. To test this hypothesis, the control and 10^{-3} M-treated plants were removed to the greenhouse. At 9:15 a.m. the plants were severed at the ground line and weighed at 15-minute intervals for 3 hours. The control plants definitely wilted faster, indicating that the $HgCl_2$ effect was on the aboveground parts of the plant.

A reevaluation of $HgCl_2$ effect on stomatal operation was made, since only mature leaves were observed in the first study (p. 44). The plants were sprayed with 10^{-4}, 0.66 by 10^{-3}, 0.33 by 10^{-3}, 10^{-3} M $HgCl_2$, or distilled water. The day after spraying, the same marked spot on each of three leaves (mature, moderately mature, and immature) of three plants of the control and 10^{-3} M treatment was observed with the RTV (room temperature vulcanizer) imprint method (*57*).

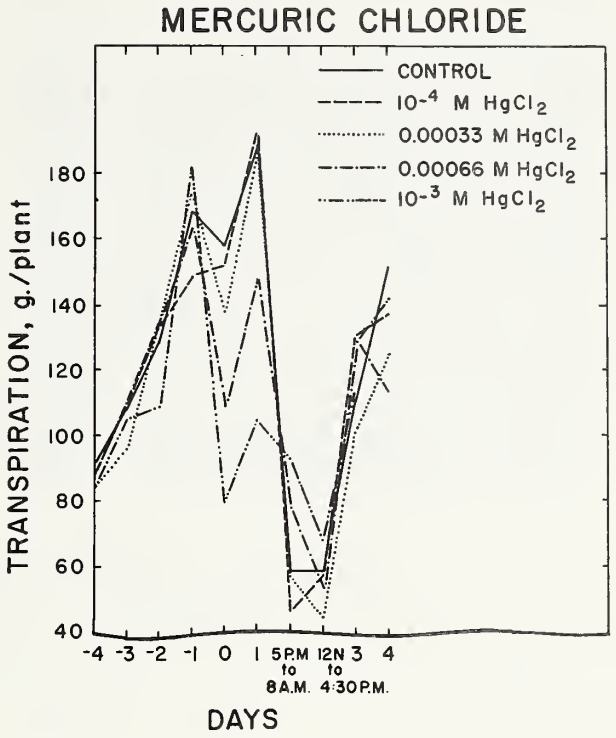

FIGURE 33.—Transpiration of red kidney bean plants sprayed at indicated concentrations with mercuric chloride ($HgCl_2$). Soil moisture stress imposed from 8 a.m. on day 1 to 4:30 p.m. on day 2.

Observations were made at 2-hour intervals beginning at 6 a.m. and ending at 8 p.m. Again no significant difference in stomatal operation could be found between treated and untreated plants. It is now assumed that the reduction in transpiration is correlated with the necrotic flecking, resulting in increased impedance to water transfer.

RTV has not been found satisfactory for extended stomatal studies. It is highly phytotoxic to both bean and corn plants under many conditions.

Phenylmercuric Acetate

Phenylmercuric chloride (C_6H_5HgCl) (10^{-4} M) has been reported to reduce transpiration of potatoes 25 percent and at the same time to increase yield 10 percent when applied four times during a 41-day experimental period (*9*). Also, phenylmercuric acetate ($C_6H_5HgOCOCH_3$) (PMA) sprayed on tobacco, corn, and sunflower plants is reported to close stomata and reduce transpiration (*59*, *60*, *80*, *81*). The reduction in transpiration ran as high as 50 percent.

The effect of PMA on the transpiration of bean plants was tested. The plants were sprayed with distilled water, 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6} M PMA. All solutions had Triton X–100 (alkyl phenoxy

polyethoxy ethanol) at 0.1 percent w/w added as a wetting agent.

The 10^{-3} M treatment was lethal. A 10- to 20-percent reduction in transpiration was recorded for the 10^{-4} M treatment (fig. 34); however, the effect lasted only a couple of days, not for an extended period as reported with tobacco and corn. A re-treatment resulted in a second drop in transpiration at only 10^{-4} M for 2 days.

PMA is already marketed as a fungicide for turf application as well as a crabgrass killer. Based on previous studies, it was considered that some benefit in moisture conservation might already exist from its regular application as a fungicide to turf. A study was undertaken to find its possible effect, if any, on the transpiration of four important bermudagrass (*Cynodon dactylon* (L.) Pers.) varieties. Sprigs of Tiffine, Tiflawn, Tifgreen, and Tifway were obtained from G. Burton at Tifton, Ga. These were planted in 3,600 gm. of the standard prepared soil. After 10 weeks of growth in the greenhouse, the cans were well sodded. They were then placed in growth chambers, and each variety was sprayed at the recommended rate for fungus control—2 or 4 ounces per 10 gallons of water per 1,000 square feet. This amounted to 7.4 ml. of the spray solution per can.

A reduction in transpiration was obtained with PMA at both concentrations on Tifgreen and Tiflawn (fig. 35). Only at the higher concentration did it appear effective on Tifway or Tiffine. No phytotoxicity was noted on any of the bermudagrasses. Whether the effect of PMA was stomatal could not be determined because of difficulty in differentiating the small, inconspicuous stomata of bermudagrass. These results indicate that PMA is effective in water conservation of turf grasses.

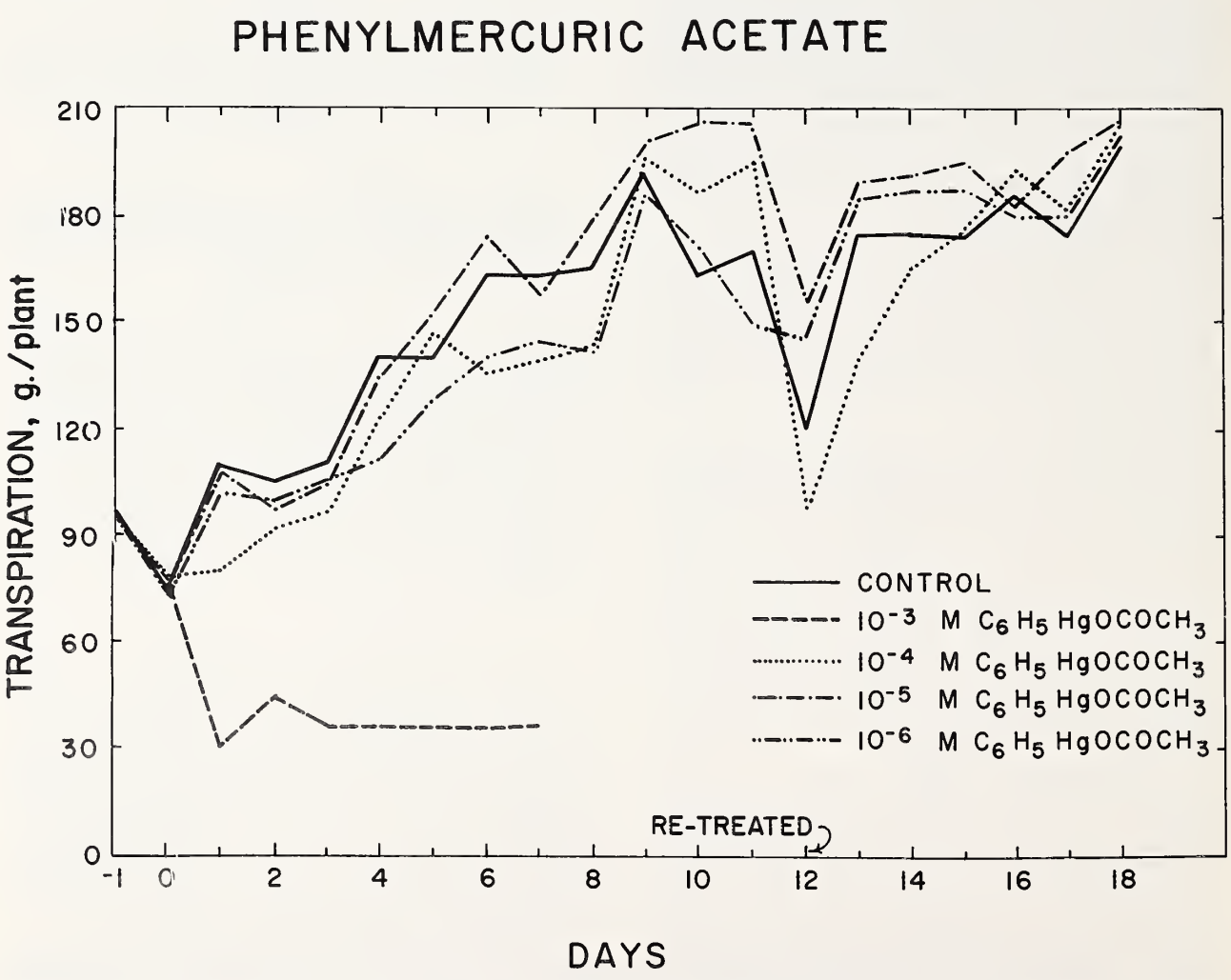

FIGURE 34.—Transpiration of red kidney bean plants sprayed at indicated concentrations with phenylmercuric acetate ($C_6H_5HgOCOCH_3$).

The mercuric ion effect could be that of an inhibitor of amylase in the guard cell, as already postulated (see p. 44), but it is difficult to reconcile the difference between $HgCl_2$ and phenylmercuric acetate found in these studies. Better penetration by the latter compound into the guard cell might explain its efficiency. At any rate the mercuric ion, being a protein coagulator, would have to be handled with caution as an antitranspirant because of possible residue. As indicated by its variable phytotoxicity in relationship to time of application as a fungicide on pears (*34*), its potential in water conservation needs very careful evaluation.[4]

[4] When this fungicide or other chemicals mentioned in this report are used in practice, proper precautions should be observed to protect humans, farm animals, and wild life as noted on the label.

α-Hydroxysulfonates

Zelitch and Waggoner (*80*) reported that α-hydroxysulfonates, inhibitors of glycolic oxidase supplied to detached tobacco leaves in sunlight, closed stomata and reduced transpiration, whereas photosynthetic carbon dioxide assimilation was not affected. Following the work of Zelitch and Waggoner and the earlier work of Odom (*46*), Stoddard and Miller (*67*) reaffirmed that 8-hydroxyquinoline sulfate does prevent wilting by affecting stomatal closure.

On the basis of Zelitch's and Waggoner's findings (*80*, *table 2*), Na α-hydroxydecanesulfonate and α-hydroxy-2-pyridylhydroxymethanesulfonic acid were two of the most effective compounds tested in causing stomatal closure. These compounds were tested by us on bean plants at the concentrations listed in figure 36. A reduction in

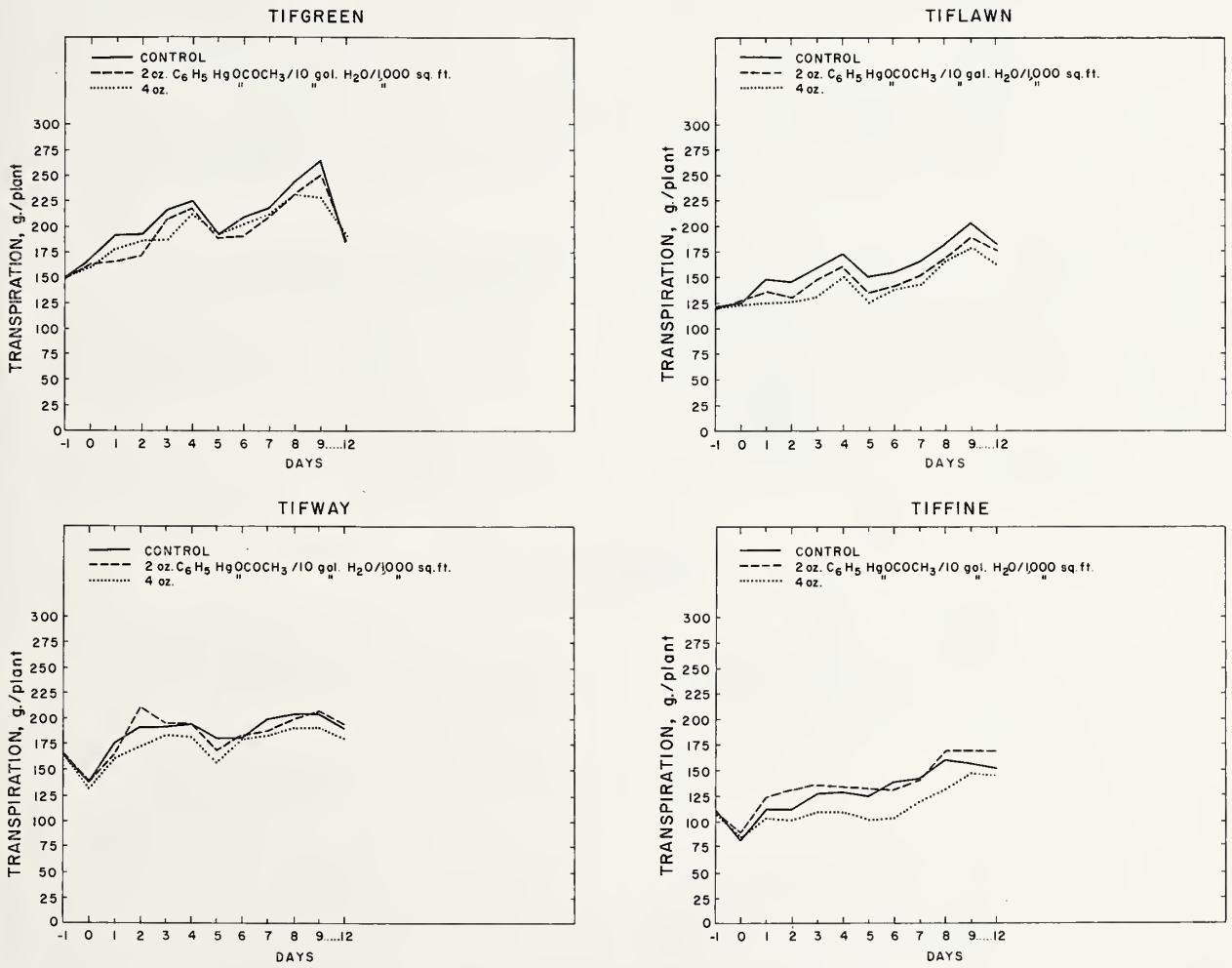

FIGURE 35.—Transpiration of four varieties of bermudagrass sprayed at indicated concentrations with phenylmercuric acetate ($C_6H_5HgOCOCH_3$).

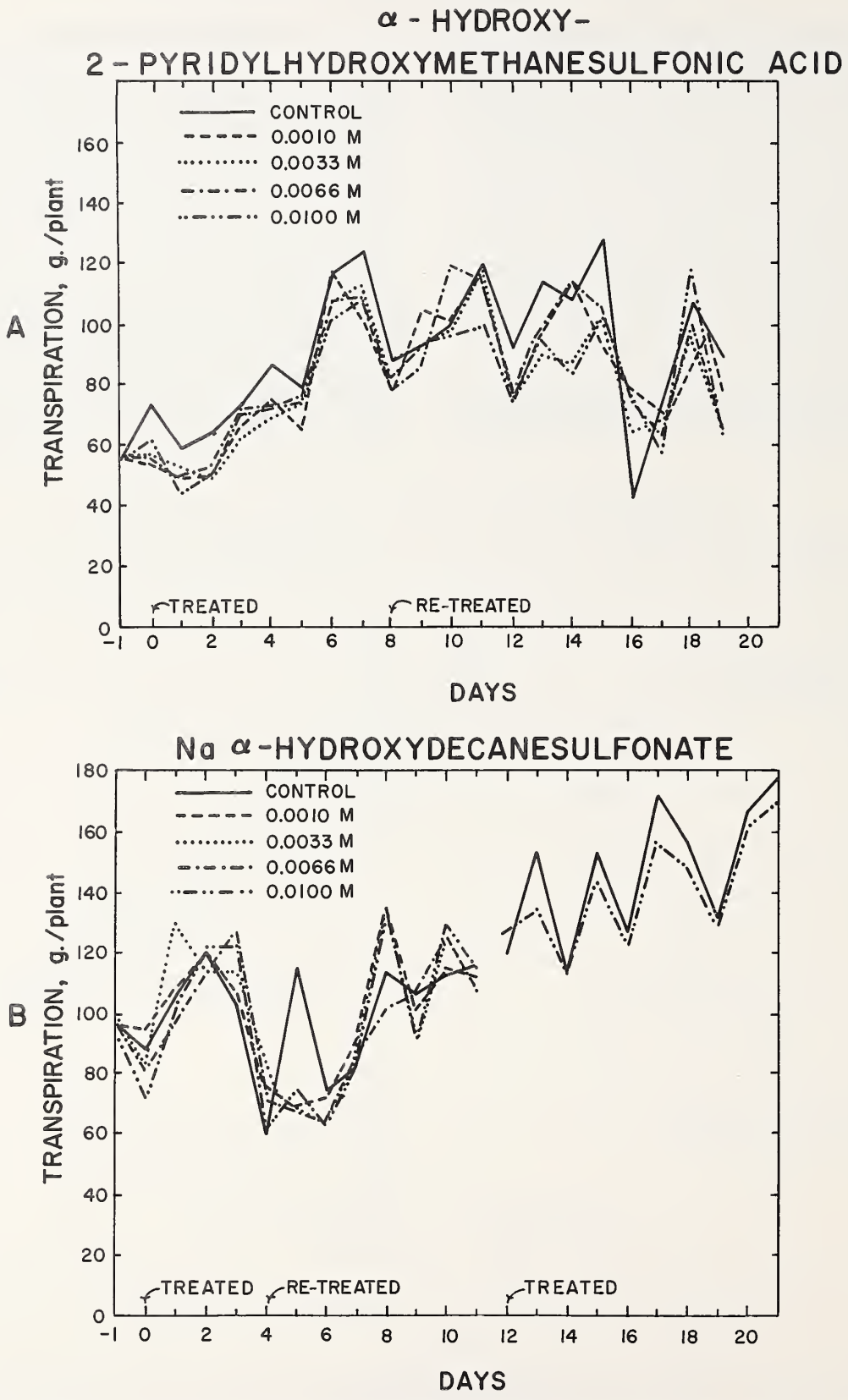

FIGURE 36.—Transpiration of red kidney bean plants sprayed at indicated concentrations with (*A*) α-hydroxy-2-pyridylhydroxymethanesulfonic acid and (*B*) Na α-hydroxydecanesulfonate.

transpiration with α-hydroxy-2-pyridylhydroxymethanesulfonic acid was evident for several days, but a 1-day effect was observed with Na α-hydroxydecanesulfonate.

Any extended field use of the sulfonates should be done cautiously, for they are effective blocks to photosynthesis (*4*). The usefulness of broad spectrum metabolic inhibitors as antitranspirants is also questionable. An example is sodium azide, already reported to give some control of guard cell operation (*64*, *79*).

Most compounds that exhibit phytotoxicity will at the same time reduce transpiration, but obviously they are not useful antitranspirants. It is evident that a lot of time and money can be used in checking the potential of major classes of chemical compounds on guard cell action and transpiration. This would be reminiscent of "shotgun" techniques that have been used for isolating herbicides, fungicides, and antibiotics. We believe that, to develop effective antitranspirants, more information must be obtained about the peculiarities of guard cell chemistry and physiology. Then those areas unique to the mechanism of guard cell action must be exploited for their potential control. If, however, developed compounds cannot be specific to guard cells, their manifold side effects must be carefully investigated and evaluated to protect the farmer and consumer.

Effect of Transpiration Suppressants on Leaf Temperature

Since transpiration was found to have a very decided effect on the leaf temperature of corn plants (p. 15), it was not surprising to observe leaf burn on plants treated with transpiration suppressants at elevated temperatures. However, to substantiate that the necrosis which developed under elevated temperatures was related to transpiration and leaf temperature, six sorghum plants, 45 days old, used in the fertilization study (p. 35) were tested.

Thermocouples were attached to the upper and lower surfaces of the third leaf from the top of each plant. Two plants were sprayed with Wilt Pruf, two with Sun Wax W, and two served as controls. The plants were watered near 0.05 atmosphere each evening to insure adequate water for the next day's studies. On the 3 successive days of the experiment, the temperature was 25°, 30°, and 35° C. and the relative humidity 60 percent. The plants received full sunlight equivalent for a photoperiod of 12 hours; the nyctoperiod was 12 hours at 20° and 90-percent relative humidity.

Figure 37 indicates the increase in leaf temperature of the treated plants. At 30° C. the temperature differential between treated and nontreated plants ran as high as 10°. Higher leaf temperatures were recorded at 35° than at 30°, but the difference between treated and nontreated leaf temperatures was not so large (approximately 6°) at the higher ambient temperature. This calls to our attention the importance of the leaf as a temperature-dependent reradiating surface. As Raschke (*55*) said, "The plant emits radiation proportional to the fourth power of its absolute temperature."

Williamson (*76*) also reported increases in leaf temperature in Wilt Pruf-treated tobacco leaves as compared to untreated leaves. Thames (*70*) reported that the survival of loblolly seedlings diminished when a wax transpiration retardant was applied. Under radiant energy of 1.1 cal. $cm.^{-2}$ $min.^{-1}$ with an ambient temperature of 78° to 80° F., waxed leaves were 4.4° above normal leaves.

In evaluating antitranspirants, potential reductions in photosynthesis must be considered as well as the effect of elevating plant temperatures.

SUMMARY

Uniformity of light distribution in the controlled environment growth room was found to be dependent on the type of bulb combination used and the size of the area. A combination of flood and spot bulbs, ratio 1 to 5, resulted in the most uniform distribution of light over an area 3 by 5 feet under each light bay.

Four experiments with corn were conducted in the controlled environmental room to determine the effect of radiant energy, temperature, relative humidity, and soil moisture tension on transpiration and guard cell activity. Data are presented to show that radiant energy, vapor pressure deficit of the air, and soil moisture tension have rather marked effects on transpiration.

The effect of radiation level on leaf temperature is very pronounced; also the effect of transpiration on lowering leaf temperature may be seen.

Guard cell operation was measurably affected by moisture availability, and the importance of a hydroactive phase in guard cell operation was indicated in the results. It appears that decreasing the free energy of water in the soil or the atmosphere may upset a delicate balance between the plant and its environment.

The dependence of photosynthesis on soil moisture availability is clearly shown. As soil moisture tension increased, photosynthesis dropped to the compensation point. Interesting interactions

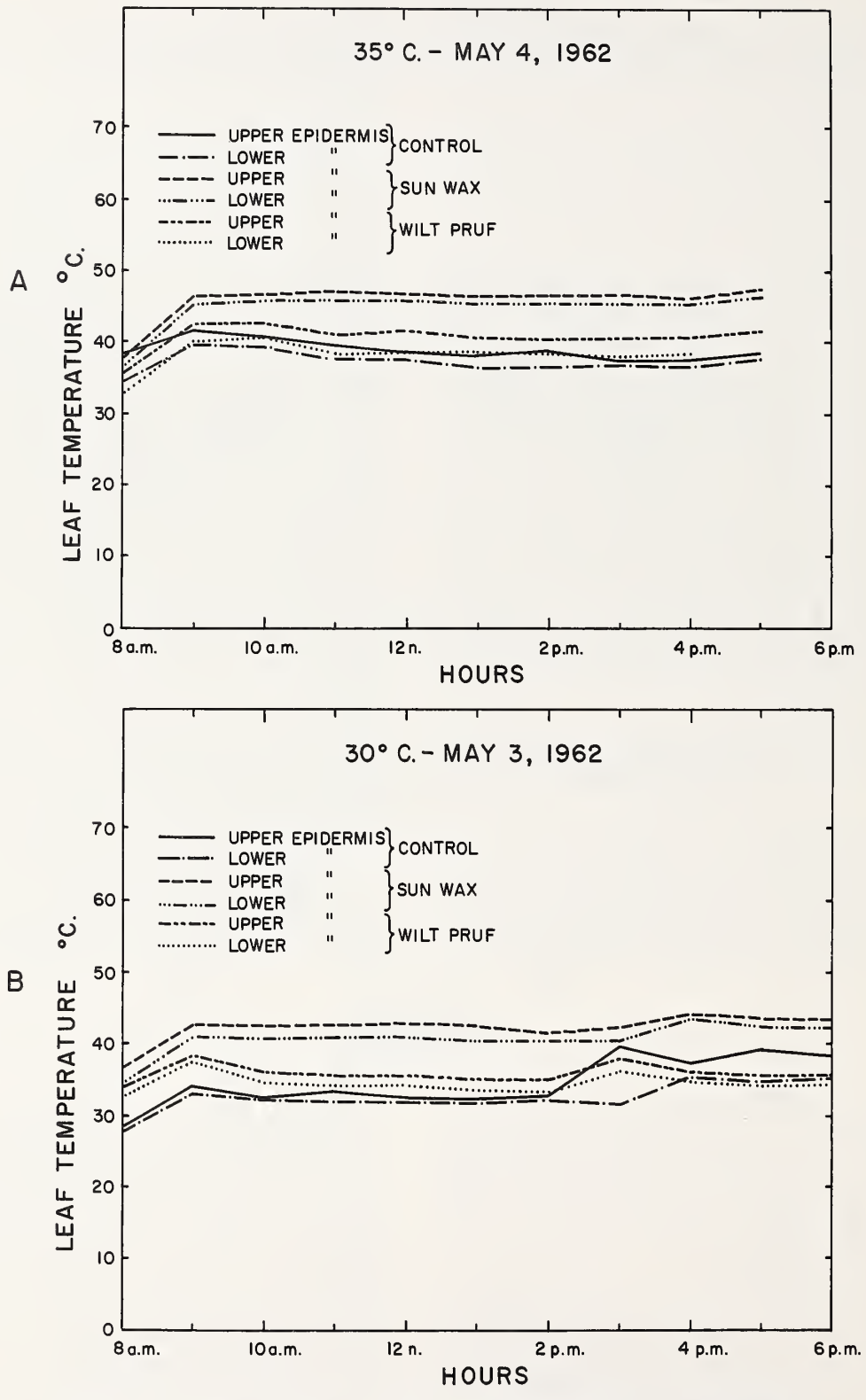

FIGURE 37.—Leaf temperatures of sorghum plants sprayed with two transpiration suppressants and a control of distilled water at indicated temperatures and 60-percent relative humidity.

between light intensity, soil moisture tension, and photosynthesis are indicated by these studies.

An evaluation of transpiration and stomatal behavior of grain sorghum under several light, humidity, and soil moisture conditions is reported. All strains studied showed a highly significant increase in transpiration at all three humidity levels when light intensity was increased. Stomatal behavior of all strains showed highly significant responses to light and humidity and to the interaction of these two variables. Stomatal activity decreased as soil moisture tension increased.

An experiment was conducted in the controlled environment room to study the effect of container size and soil fertility level on transpiration, growth, and nutrient uptake of sorghum plants. Dry-matter production was greater in the larger volume of soil. Plant growth increased as the rate of phosphorus added was increased and decreased as the rate of potassium chloride added was increased. High rates of the latter resulted in reduced root growth.

Studies of the osmotic pressure relationships of guard cells revealed that, with the species studied, opening and closing movements of stomata could not be unequivocally correlated with osmotic pressure changes.

Compounds evaluated as transpiration suppressants included several latex and plastic compounds, waxes, mercury and fluoride compounds, and α-hydroysulfonates. Most of them did not reduce transpiration without also depressing plant growth. In some cases temperature of treated leaves was elevated to the "kill point" when the plants were placed under high light.

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APPENDIX

Bulb Arrangement for Light Distribution Study

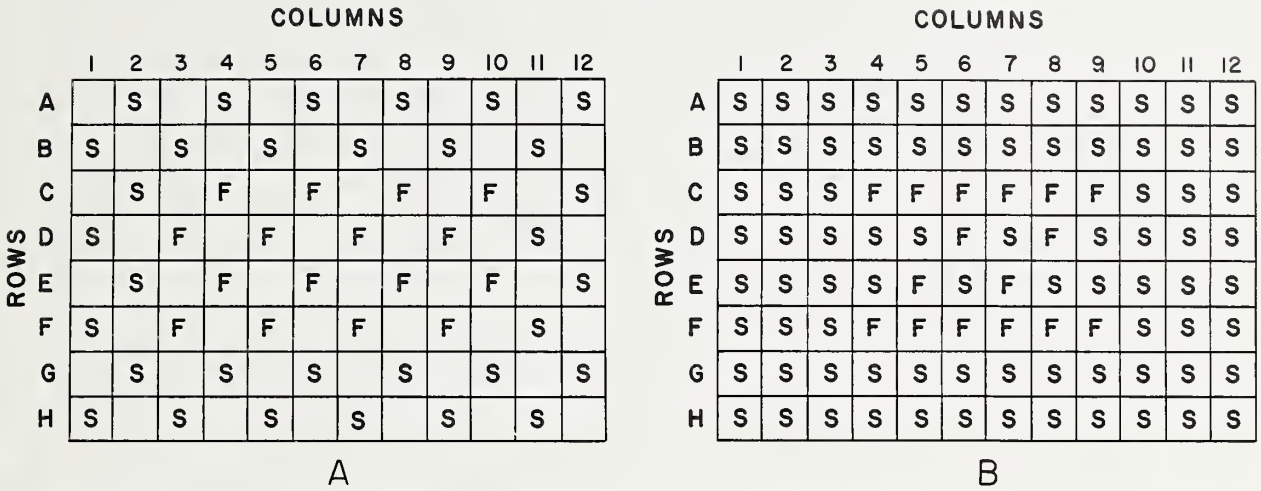

FIGURE 38.—Arrangement of 300-watt reflector bulbs used in light distribution study (table 1): *A*, Code 6, 32 spot (S) and 16 flood (F) bulbs; *B*, code 7, 80 spot and 16 flood bulbs.

Transpiration and Stomatal Data

TABLE 13.—*Table of variables correlated with hourly transpiration rates (E_h)*

Variable	Variable	Variable
(1) R_h	(14) t·L	(27) 1nB·L
(2) R_h·L	(15) t^2	(28) L/1nB
(3) R_h·1nL	(16) t^2·L	(29) R_h·V·L
(4) V	(17) B	(30) R_h·T·L
(5) V·L	(18) B^2	(31) R_h·t·L
(6) V·1nL	(19) 1nB	(32) R·B·L
(7) T	(20) B·L	(33) V·T·L
(8) T·L	(21) B^2L	(34) V·t·L
(9) T·1nL	(22) B^{-1}	(35) V·B·L
(10) T^2	(23) B^{-2}	(36) t·B·L
(11) T^2·L	(24) 1/1nB	(37) L
(12) T^2·1nL	(25) B^{-1}·L	(38) S_l
(13) t	(26) B^{-2}·L	(39) S_u

NOTE: Symbols used in identification of variables in correlation analysis:

E_h=transpiration loss in grams per plant per hour (gm. $plant^{-1}$ $hr.^{-1}$).

R_h=radiant energy in calories per square centimeter per hour (cal. $cm.^{-2}$ $hr.^{-1}$).

L=leaf area of plant in square decimeters per plant ($dm.^{-2}$ $plant^{-1}$).

V=average vapor pressure difference of water in air in millibars (mb.).

T=average air temperature in degrees centigrade (° C.).

t=temperature gradient between leaf temperature and air temperature in degrees centigrade (Leaf temperature—air temperature=t ° C.).

B=estimated soil moisture tension in bars (b.).

S_l=percent of lower stomata open.

S_u=percent of upper stomata open.

TABLE 14.—*Analysis of variance of stomatal activity (day 7)*

Item	Degrees of freedom	Sum of squares	Mean of squares	*F*
Bay	2	3204. 6990	1602. 3495	12. 70
Period	9	2420. 0960	268. 8998	2. 13
B×P	18	2258. 0760	125. 4487	N.S.
Error	30	3784. 9135	126. 1638	

Latex and Plastic Compounds

The following information was provided by the respective manufacturer:

Wilt Pruf

The material increases safeness of transplanting tobacco, tomatoes, and various perennials. Specific instances wherein the compound has increased water conservation are as follows:

(1) Protected turf during drought in Southwestern States and Montana.

(2) Prevented needle drop and preserved Christmas trees (Norway spruce).

(3) Protected propagated cuttings and buds in budding.

(4) Kept evergreen branches fresh for floral arrangements.

(5) Decreased winterkill of roses, Pfitzers, pinion pines, Austrian pine, Mugho pine, *Juniperus scopulorum* Sarg., and *Tamarix juniperina* Bge.

(6) Treated tomato plants had 89-percent survival rate as compared to 25-percent for untreated plants.

(7) Protected dahlias and other tubers in storage from drying out.

(8) Made possible the transplanting of 40- to 50-foot pine and oak trees.

Rutex W–3

The material is recommended for woody plants but not for succulents. It is not a substitute for wax on packaged roses nor will it prevent winterkill. The company also markets Rutex 59 for application to roots of plants in dormant storage, bare root shipping, and transplanting. Suggested uses of the material are as follows:

(1) To prevent excessive dehydration from dry winter and hot summer winds.

(2) To reduce afternoon wilt and maintain freshly watered appearance of leaves.

(3) To combat excessive transpiration before and after transplanting.

(4) To prolong life of cut flowers and foliage.

(5) To increase storage life of bulbs.

Vanex

Specific instances wherein the compound has increased water conservation are as follows:

(1) Improved survival of bush honeysuckle, huckleberry, blueberry, rhododendrons, rose of Sharon, magnolia, apples, peach, pear, cherry, maple, birch, and oak.

(2) Reduced transplanting setback of evergreens.

(3) Lessened winter injury by fall spray.

(4) Enhanced appearance and maintained vitality until planting of bare root roses (1:3 to 1:9).

(5) Decreased injury of transplanted tomato (1:9).

(6) Increased storage life of dormant deciduous trees (1:4).

(7) Prolonged life of cut flowers and greens.

(8) Increased storage life of tulip, hyacinth, and gladiolus bulbs.

(9) Reduced weight loss and spoilage during storage and shipment of citrus fruit, sweet potatoes, tomatoes, squash, and apples.

Latex 5229 and 5230 (Experimental)

Manufacturer gave tabular data showing a 64-percent reduction in transpiration from excised tomato leaves by Latex 5229 and 68-percent by Latex 5230 (1:6 v/v).

U.S. GOVERNMENT PRINTING OFFICE: 1965 O—754–939

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RESEARCH IN PLANT TRANSPIRATION: 1962, by James E. Pallas, Jr., Anson R. Bertrand, Donald G. Harris, Charles B. Elkins, Jr., and Clyde L. Parks. U.S. Dept. Agr. Prod. Res. Rpt. 87.

Annual Report (1 July 61 through 30 June 62)

Pub. Jan. 1965, ERDAA Technical Program, DA Task 3A99-27-005-08

56 p. incl. illus., tables, 82 refs. Unclassified Report.

Experiments were conducted to measure plant transpiration in a controlled environment growth room. Radiant energy, relative humidity, and soil moisture tension had marked effects on the transpiration rate.

Guard cell operation was measurably affected hy moisture availahility.

Osmotic pressure determinations made concurrently with stomatal ohservations showed that, with the species studied, operation of stomata could not hc correlated with changes in osmotic pressure of the guard cells.

Tests were made of several formulations applied to leaves to provide either a physical harrier to transpiration or a potential control of stomatal operation. Materials included latex and plastic compounds, waxes, mercury and fluoride compounds, and α-hydroxysulfonates. Although a numher of these formulations reduced transpiration, few did so without also depressing plant growth. In experiments with the most effective transpiration suppressants, treated leaves developed a temperature differential of 6° to 10° C. over that of untreated leaves.

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